POSTHARVEST PHYSIOLOGY OF RED GINGER INFLORESCENCE
(*Alpinia purpurata* Vieill K. Schum)

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ABSTRACT

The postharvest physiology of red ginger inflorescence, including the impact of heat treatment to extend inflorescence vase life, was determined. More than 90% of the inflorescences showed inflorescence wilting or bract browning symptoms or both during senescence. Ethylene and the total count microorganisms in the stem segments and the vase solution had no effect on the development of senescence symptoms. I hypothesized that water balance of the cut stem was associated with inflorescence wilting, whereas sugar content was associated with bract browning symptom. While a relationship between inflorescence wilting and water balance was not confirmed, a positive relationship was established between sugar content and inflorescence vase life as the higher the sugar content in cut stem, the longer the inflorescence vase life.

Immersion of the inflorescences in hot water at 40°C for 15 min is recommended as a preconditioning treatment to prevent heat damage to red ginger from the hot water treatment at 50°C for 12 min for insect disinfestation. This combination treatment extends vase life. Extension of inflorescence vase life by the hot water treatment varied with season of harvest and flower variety. Exposure time shorter than 12 min at 50°C was recommended in winter, as the inflorescences had lower thermotolerance than in the summer.

Hot water treated inflorescences exhibited a lower rate of respiration than untreated inflorescence, while ethylene production as a result of the treatment was not significantly detected. Inflorescences treated with hot water maintained a higher sugar

levels for a longer period than untreated inflorescences, and this could explain the vase life extension. Hot water treatment also suppressed negative geotropism in the red ginger inflorescences during horizontal storage. The negative geotropic response was delayed for up to 7 days after the hot water treatment.

The sugar content (98%) in the red ginger stem was located in symplast, whereas two percent was attributed to apoplast that is heat resistant as the contents were not affected by the hot water treatment. Sugar metabolism in red ginger inflorescence may be heat sensitive. However, the activity of sugar metabolic enzymes: sucrose phosphate synthase, sucrose synthase and invertase, were not directly related to the changes in sugar content after hot water treatment. It is, therefore, suggested that factors other than the activity of these enzymes affect sugar content of the cut stem.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Red ginger (*Alpinia purpurata* Vieill K. Schum) is commonly used as an ornamental plant as it is easily grown and has attractive red bracts. However, references to red ginger inflorescence physiology are rare. Occasionally, postharvest handling methods, i.e. preservative and holding solutions are reported (Broschat and Donselman, 1988), whereas postharvest physiological changes associated with inflorescence senescence, i.e. ethylene production, respiration rate, energy source, and water balance of cut stem have not been studied. In order to develop postharvest handling treatment and understand the mechanisms by which vase life is extended, changes in postharvest physiology of cut inflorescences, including major factors associated with inflorescence senescence, need to be determined.

The ginger inflorescence is a complex organ composed of many morphological units such as petals, sepals, androecium, gynoecium, leaf, colored bract, and main stem or rachis (Dahlgren *et al.*, 1985). The interaction between these organs may have some effect on flower longevity. Postharvest factors associated with cut flower senescence include plant growth regulators, water balance of cut stems and level of energy resources (Halevy and Mayak, 1981).

Ethylene is the most important plant growth regulator that triggers senescence symptoms in several cut flowers, especially as a post-pollination response in orchids (Bui

and O'Neill, 1998) and carnation (Jones and Woodson, 1997). Senescent symptoms caused by ethylene include inrolling, fading and wilting of flower organs which can be used to assay the sensitivity of plants to exogenous ethylene (Gilissen, 1977). However, the effect of ethylene on red ginger senescence has never been documented. Cytokinin is another plant growth regulator associated with delay of leaf senescence (Dumbroff and Walker, 1979; van Staden *et al.*, 1988). The exogenous application of benzyladenine (BA) has been reported to delay senescent symptoms in cut rose (Lukaszewska *et al.*, 1994), Easter lily leaves (Han, 1995) and broccoli (Downs *et al.*, 1997). This response is possible in the bracts of the red ginger as they are modified leaves.

Differences between water uptake and water loss have been reported as a cause of stem breakage in cut gerbera (van Meeteren, 1978a) and bent neck in cut rose (Burdett, 1970). While water loss from cut flower stems is mainly as transpiration via stomata, water uptake is affected by several factors such as growth of microorganisms, physiological products from the cut stem and air embolism in vascular tissues (van Doorn, 1989). However, cut flower senescence could occur without a significant decrease in water uptake and water potential (van Doorn *et al.*, 1995a) suggesting other factors are associated with flower senescence.

The energy supply for a cut flower, mainly carbohydrate, has been associated with senescence of some cut flower and foliage such as in gladiolus (Kofranek and Halevy, 1976) and protea leaf blackening (Dai and Paull, 1995). This occurs as the flower, once cut from the mother plant, no longer has a ready source of water, hormone, photosynthates and other nutrients (Paull, 1991). Therefore, the flower must rely on its

own energy resources. Hence, application of exogenous sugar extends flower vase life (Brink and Swardt, 1986; Doi and Reid, 1995; Kofranek and Halevy, 1976). However, wilting can occur without depletion of flower energy levels (Nichols, 1973b). Factors associated with flower senescence, therefore, vary with plant species and more over, with senescence symptoms. While wilting is associated with ethylene production and water imbalance of the cut stem, blackening or discoloration symptoms could result from depletion of energy resources.

Senescence in the red ginger may be associated with a reduction in water uptake due to the plugging of the vascular tissues (Paull, 1991). Red ginger senescence could be the result of the low energy sources of the cut stem, because the application of exogenous sugar can extend flower vase life (Broschat and Donselman, 1988; Tjia, 1988). However, any postharvest change in red ginger endogenous sugar level has not yet been determined.

Preparation of red ginger for packing requires labor-intensive hand washing and dipping in insecticidal solutions. Hot water treatments to control quarantine insects in tropical cut flowers and foliage have been developed (Hara *et al.*, 1993; Hara *et al.*, 1996; Tenbrink *et al.*, 1992). Although, hot water treatment (49°C, 12 min (Hara *et al.*, 1996)) effectively kills the target insect species and extend red gingers vase life, additional study is required to determine if this is optimum for vase life extension and to reduce inflorescence damage. For example, the threshold limitation for hot water treatment causing inflorescence damage is unknown and appropriate preconditioning treatments

that can reduce inflorescence damage have not been reported. Furthermore, the mechanism by which hot water extends inflorescence vase life has not been determined.

The previous studies allow the formation of a hypothesis: that the senescence of red ginger is associated with either or both the depletion of energy sources or the water balance of the cut stem rather than ethylene production or the tissue sensitivity to the ethylene. Hot water treatment may extend inflorescence vase life by altering the level of sugars within the cut stem and may affect activities of enzymes involved in sugar metabolism. The objectives for these studies are:

1. Determine the pattern of changes in postharvest physiology associated with inflorescence senescence. In this study, 200 ppm benzyladenine (BA) was used to alter inflorescence senescence.

2. Determine suitable temperatures and exposure times of the hot water treatment to prolong vase life of the red ginger including the preconditioning treatments to reduce damage of the hot water treatment and the effect of selective treatments on inflorescence vase life.

3. Determine the effect of the hot water treatment on postharvest physiology of red ginger inflorescences.

4. Determine the effect of hot water treatments on carbohydrate resource and the activity of sucrose metabolic enzymes in inflorescence bracts and cut stem sections.

The above objectives were addressed by the following studies:

1. Senescence development and inflorescence characteristics. The observation of the development of senescence symptoms, classification of the symptoms and the

postharvest inflorescence characteristics, including postharvest handling system were determined.

2. Changes in postharvest physiology during senescence. Ethylene production, respiration rate, water uptake, water loss rate, number of total count microorganisms, and levels of starch and sugars were determined. All parameters were monitored from harvest until the end of vase life. The pattern of changes in these parameters was expected to allow a conclusion as to the major parameters related to red ginger senescence.

3. Hot water treatments. The relationship between temperature and exposure time on inflorescence vase life, the effect of preconditioning treatment to reduce damage, and the relationship between preharvest factors on bract damage and inflorescence vase life were determined. This study should allow the recommendation of an appropriate treatment to extend vase life and the suitable preconditioning treatment to reduce bract damage including the manipulation of the treatments within a year.

4. Effect of hot water treatment on postharvest physiological factors. This study concentrated on the respiration rate and ethylene production, water uptake and water loss rate, total count of microorganisms, and level of energy reserves. This study should provide an understanding of the mechanism by which hot water maintains vase life and inflorescence quality.

5. Effect of hot water treatment on energy reserves and enzyme activity. Included in this study were sugar components, location of sugars and the activity of enzymes

involving in sugar metabolism. This study was expected to give an understanding of the importance of carbohydrate supply on inflorescence vase life during senescence.

1.2. Red ginger (*Alpinia purpurata* Vieill K. Schum)

1.2.1. Summary of botany and cultivation

Red ginger (*Alpinia purpurata* Vieill K. Schum) belongs to family Zingiberaceae and has a banana-like habit being perennial and rhizomatous (Dahlgren *et al.*, 1985). The ginger family contains at least 1400 species and perhaps even as many as 2000 (Chapman, 1995). The sympodial rhizome is generally horizontal and creeping, often thick and rich in starch. The vessels have scalariform perforation plates. The leaf sheaths are often long and superimposed to form a pseudostem like that in *Musa*. Unlike banana whose inflorescence-bearing shoot emerges directly from rhizome, the *Alpinia* leafy branch terminates in an inflorescence. The inflorescence has leaves that consist of scale-like sheath known as bracts. Unlike the leaves of the vegetative part, the bracts of the inflorescence are spirally set and colorful. The true flower is inconspicuous, white and tubular and is less spectacular than the colorful bracts (Criley and Paull, 1993).

Red ginger is native to French Polynesia and other Pacific Islands and widely cultivated as an ornamental from Thailand to Micronesia, Melanesia, Polynesia and extending across the Pacific to Venezuela, Colombia, Honduras and the Caribbean (Griffith, 1984; Hirano, 1991). Gingers are popular as they are easily grown, especially in the tropics. Most gingers grow well in rich, well-drained soil, while a few love wet conditions (Chapman, 1995). In-row spacing of 1.2 to 2 meter is recommend (Criley,

1989) and all-purpose fertilizers, such as 15-15-15, work well. Propagation can be achieved either by rhizome division or rooting of the aerial offshoot. Recent discovery of seed set has lead to new color forms (Hirano, 1991). Inflorescence can be harvested within a year of re-establishment by rhizome division, but at least 2 years are required for plantlets from aerial offshoots to produce marketable inflorescences (Criley, 1989).

1.2.2. Vascular system

The fundamental feature underlying monocotyledon vascular system appears to be the 'upward-branching leaf trace' (Zimmermann and Tomlinson, 1972) with the axial bundles running along the length of the stem gradually approaching the stem center and then turning outward.

In shell ginger (*Alpinia speciosa*), the vascular tissue of scattered vascular bundles in aerial axis is arranged in two distinct zones: an *inner* and *outer* system, separated by a cylinder of undifferentiated tissue that retains the features of a meristem (Bell, 1980a; Bell, 1980b). The inner system is found to have the simple basic design of a typical palm stem i.e., a system of upwardly branching leaf trace with interconnections whereas, the outer system is considered to be equivalent to the non-conducting fibrous bundles present at the peripheral of many palm stems. There is no bridge or fine connection between inner system bundles. Unlike the system in palms where the bundle splits into branches before entering the leaf base (Zimmermann and Tomlinson, 1972), bundle in *Alpinia* departs as a whole for inner system leaf trace. Outer system bundles frequently split and rejoin to form many bridges resulting in an elaborate network. Due to the comparable

large size of the cortical bundles both in the stem and leaf sheath, together with the dependence of the inner system on the outer, it has been suggested that the outer system is functionally more important in *Alpinia* (Bell, 1980b).

1.2.3. Postharvest handling

Inflorescences are normally cut at the two-third or three-fourth open stage. The two-third opening stage refers to the two-thirds of the inflorescence bracts reflectively open from the rachis to reveal the tubular white flower inside. The inflorescences are normally packed together with other tropical flowers and foliage (Broschat and Donselman, 1988). To prevent drying during shipping, bunches may be wrapped in a plastic sleeve and packed with moistened shredded newspaper (Criley and Paull, 1993). Due to its sensitivity to chilling injury, red ginger is shipped at temperatures not lower than 12°C and should be stored vertically in order to prevent inflorescence bending due to geotropism (Criley and Paull, 1993). The major symptoms of senescence in the red ginger are the inrolling of the leaves and darkening of the bracts, which may be associated with a reduction in water uptake due to the plugging of the vascular tissues (Paull, 1991). Vase life of the red ginger is reported to be strongly affected by stem length and diameter: the larger the inflorescence, the longer the vase life (Broschat and Donselman, 1988).

While pulsing red ginger with 2% sucrose and 800 ppm 8-HQC for 4 h (Broschat and Donselman, 1988) does not extend inflorescence vase life, holding solutions containing 2% sucrose and 200 ppm 8-HQC extend vase life about 2 fold (Broschat and

Donselman, 1988; Tjia, 1988). Pulsing red ginger stem with 2 mM silver thiosulfate (STS) for 4 h causes phytotoxicity and reduces inflorescence vase life by about one-third (Broschat and Donselman, 1988). Acidifying agents such as citric acid (5 μM) or amino-oxy acetic acid (AOA, 25 μM) significantly increase vase life. The vase life of the inflorescence increases nearly three fold with citric acid alone (Tjia, 1988). Although the function of acidifying agents on postharvest vase life of cut flower is unknown, part of its function is thought to control bacterial growth in the vase solution (Marousky, 1971). Microorganisms in the vase solution seem to play a role in postharvest vase life of red ginger since simply recutting of the stem before placing into water almost doubles inflorescence vase life compared to not recutting (Broschat and Donselman, 1988). This is possibly due to the cut end of the stem being a major site for microbial growth (van Doorn *et al.*, 1989). Therefore, recutting the stem is a simple means to remove the site of microbial accumulation, hence extending inflorescence vase life.

1.3. Postharvest physiology of cut flower senescence

1.3.1. Ethylene production and respiration rate

The role of ethylene in flower senescence has been well documented (Burg, 1962; Halevy and Mayak, 1981; Nadeau *et al.*, 1993; Woodson *et al.*, 1992). The senescence symptoms associated with ethylene production included: inrolling of the petals, fading and inrolling of collar, fading and wilting of sepal lips and the abscission of the flowers and petals. These symptoms are well defined and normally used to assay the sensitivity of plants to exogenous ethylene i.e., petunia (Gilissen, 1977), gypsophila (van Doorn and

Reid, 1992), and other foliage plants (Woltering, 1986). The changes in ethylene production during flower senescence vary with plant species and cultivars. In *Gladiolus* sp., only 'New Rose' and 'Nancy' cultivars show ethylene production in a climacteric-like pattern, whereas 'St. Patrick', 'Captain Busch', 'Fond Memory', and 'Blue Mountain', do not show an obvious climacteric pattern (Serek *et al.*, 1994a). Tissue sensitivity to ethylene varies with plant species and stages of flower development. For example, *Dendrobium* 'Pompadour' ethylene sensitivity increase as the flower develops from the immature to the fully mature stage (Nair and Fong, 1987), whereas carnation 'Sandrosa', a non-autocatalytic ethylene production variety, shows declining sensitivity to ethylene with maturity (Mayak and Tirosh, 1993).

It has been well documented that pollination is the major cause for ethylene production leading to petal senescence (Bui and O'Neill, 1998; Jones and Woodson, 1997; O'Neill *et al.*, 1993). However, the signal for inter-organ communication has not been unequivocally determined. While 1-aminocyclopropane-1-carboxylic acid (ACC) was reported to be translocated among interorgans of *Phalaenopsis* orchid and induce ethylene production (Bui and O'Neill, 1998), the same translocation has not been found in petunia flowers (Woltering *et al.*, 1997). Postharvest application of antiethylene synthesis and action helps to prevent or delay the ethylene-induced senescence in several cut flowers. Chemicals used to prevent ethylene action by acting as competitive inhibitors include silver thiosulfate (STS) (Cameron and Reid, 1983; Staby and Naegele, 1984), norbornadiene (Wang and Woodson, 1989), and 1-methylecyclopropene (MCP) (Serek *et al.*, 1994b; Serek *et al.*, 1994c; Serek *et al.*, 1995). Ethylene biosynthesis

inhibitors that inhibit the synthesis of ethylene precursor, 1-acimocyclopropane-1-carboxylic acid (ACC) include aminooxy acetic acid (AOA) and aminoethoxyvinyl glycine (AVG) (Halevy and Mayak, 1981).

In general, the postharvest respiration rate of cut flowers declines, but may increase at the end of vase life (Coorts, 1973). The increase is normally associated with the development of senescent symptoms, such as in anthurium (Paull *et al.*, 1985) and Easter lily leaf (Franco and Han, 1997). The limitation of respiratory substrates is suggested as a cause for the decrease in respiration rate (Coorts, 1973; Marousky, 1969). The rise in respiration rate at the final stage of senescence could be caused by a disruption of the membrane structure and cytoplasmic organelles in the petal cells (Nichols, 1973b). However, a limitation in respiratory substrate is not always the cause for flower senescence. Indeed, incipient wilting of rose and carnation petals occurs prior to the exhaustion of petal carbohydrate supply (Kaltaler and Steponkus, 1976; Nichols, 1973b). Therefore, the function of sugars as a respiratory substrate might vary from species to species. In cut rose, sucrose in holding solution seems to maintain mitochondrial structure and function rather than providing respiratory substrate (Kaltaler and Steponkus, 1976). Information on ethylene production and respiration rate is not available for red ginger.

1.3.2. Water balance of the cut stems

Cut flower fresh weight change is determined by two components: water uptake and water loss. Subtracting rate of water loss (transpiration) from rate of water uptake is

used to determine water balance of the cut stem (van Doorn *et al.*, 1995b). In cut rose, loss of petal turgidity and fresh weight is preceded by a decrease in the rate of water uptake, indicating that reduced uptake rather than excessive water loss is responsible for rose senescence (Durkin and Kuc, 1966).

Postharvest rate of solution uptake can be induced by low pH of the holding solution, i.e. pH 3 to 4, although its function on water uptake has not been clear (Conrado *et al.*, 1980). However, a higher rate of water uptake induced by lower pH of solution is not always correlated with longer vase life. Optimal solution uptake and the optimum solution composition to extend vase life occur at different pHs. For example, in cut roses, optimum solution uptake occurs at pH 2, whereas optimal vase life occurs with a pH 5 solution (Conrado *et al.*, 1980). This finding suggests that the pH of the vase solution is only one of several factors affecting the flower longevity. Water loss rates of Dendrobium flower sprays cv. 'Princess' (*Dendrobium spp.*) declined from 1.25 $g \cdot day^{-1} \cdot spray^{-1}$ (4 days after harvest), to 0. 35 $g \cdot day^{-1} \cdot spray^{-1}$ (20 days after harvest), with flower shedding when the rate of water loss fell below 1.0 $g \cdot day^{-1} \cdot spray^{-1}$ (Dai and Paull, 1991). In red ginger, although several preservatives have been reported to extend vase life (Broschat and Donselman, 1988; Tjia, 1988), the water status of the cut stem has never been documented.

1.3.3. Vascular blockage of cut flowers

There are three major possible causes of vascular blockage in a cut flower stem: air in the vessel, products from physiological process, and the growth of microorganisms

(Halevy and Mayak, 1981; van Doorn, 1989). Air embolism at the stem base affects the rate of water uptake and its effect is dependent on the time that flowers are exposed to air during dry storage. Keeping cut rose in air for up to 3 hours did not affect subsequent rate of water uptake (van Doorn, 1989). Air embolism after dry storage can be prevented by recutting the stem under water (Parups and Molnar, 1972). Blockage from physiological process was thought to be a plant responded to wounding. In cut rose, the gum occlusion above the solution level results from redeposition of existing gum components rather than *de novo* synthesis of those components (Lineberger and Steponkus, 1976). The occlusive materials have been reported to be pectinaceous material (Parups and Molnar, 1972). The vascular blockage from either or both the growth of microorganism or their released product is mostly found at the cut end of the stems (Lineberger and Steponkus, 1976).

1.3.4. Microorganisms in cut flowers

The presence of microorganisms, mostly in the lowest 1 cm of cut stem, and their metabolites frequently lead to vascular blockage in cut flowers (Marousky, 1980; van Doorn *et al.*, 1995b; Zagory and Reid, 1986a; Zagory and Reid, 1986b). At high bacterial concentrations, 3x 10^9 cfu·ml^{-1} (van Doorn *et al.*, 1986), water uptake and transpiration of cut rose declines within an hour and bent-neck symptoms are observed. Microorganisms involved in vascular blockage of cut flowers include bacteria, pseudomonads and yeasts (van Doorn *et al.*, 1995b; Zagory and Reid, 1986a; Zagory and Reid, 1986b). Since vessel blockage may occur under sterile condition (Marousky, 1969), microbial growth is not the only cause for vascular blockage.

Quinoline salts i.e., 8-hydroxyquinoline citrate (8-HQC) are known to be acidifying as well as fungistatic, inhibiting many fungi and bacteria (Larsen and Cromarty, 1967; Marousky, 1971; Marousky, 1980). Although, low pH is reported to be one of major factors enhancing cut flower life by enhancing water conductivity (Durkin, 1979) and controlling microorganism growth (Marousky, 1971), the number of different microbes in the vase solution are not affected by pH of holding solution (Zagory and Reid, 1986b). The discrepancy in these reports may result from the difference in experimental set up. While Marousky (1971) reported on general effect of low pH as to control total microorganisms, Zagory and Reid (1986b) studied only on specific species. For dendrobium sprays, postharvest vase life is extended by using boiled deionized water and by the continuous presence of, whereas other antimicrobial agents such as sodium hypochlorite, thiabendazole, Physan-20, sodium dichloro-s-triazinetrione dihydrate, and sodium dichloro-s-triazinetrione dihydrate, have no effect on dendrobium vase life (Dai and Paull, 1991).

1.3.5. Energy sources of cut flowers

Sugar levels: The sugar content inside the cut stem and floral tissues is one factor determining flower longevity (Halevy and Mayak, 1979). Cutting stems from the mother plant also removes the sources of nutrients, photosynthates, hormones and water (Paull, 1991), hence cut flowers routinely deteriorate much more quickly than flowers left on the plant under similar environmental conditions. Reducing sugars, rather than sucrose, are reported as the main constituents of the sugar pool in the mature petals of several species

(Halevy and Mayak, 1979; Ho and Nichols, 1977; Kaltaler and Steponkus, 1974; Nichols, 1973b) and this supports the view that the floral tissues are active metabolic centers. The major transported sugar inside cut flower stem are thought to be mainly sucrose (Kaltaler and Steponkus, 1974; Sacalis and Chin, 1976), whereas in certain species sugar alcohols or oligosaccharides may be involved (Kruger, 1990).

The levels of sugars in flower petals vary with plant species, developmental stages, and plant tissues. At harvest, reducing sugar has been reported to be about 2 to 3 % of the fresh weight in cut carnation (Nichols, 1973b), and about 200 to 300 $\mu moles \cdot g^{-1}$ petal tissue in cut rose (Kaltaler and Steponkus, 1974). Sucrose is present in the flower petals at a lower level than reducing sugar which major forms found in petals are glucose and fructose (Nichols, 1973a). For example, 0.3 to 1.0 % of the fresh weight of carnation (Nichols, 1973b) and less than 23 $\mu moles \cdot g^{-1}$ petal tissue in rose (Kaltaler and Steponkus, 1974). In narcissus, sucrose is the predominant sugar in the corolla when the flower is harvested at the bud stage, but, when the flower opens, sucrose rapidly disappears concomitant with an increase in reducing sugar accumulation (Nichols, 1973a). The accumulation of reducing sugar during flower development after harvest is also reported for *Sandersonia aurantiaca* (Eason *et al.*, 1997). The levels of sugar in floral tissues decrease with time after harvest (Ho and Nichols, 1977; Kaltaler and Steponkus, 1974; Nichols, 1973b) or after reaching a maximum during the senescence process (Eason *et al.*, 1997; Nichols, 1973a). This finding supports the view that the limitation of respiratory substrate is another factor influencing flower vase life. However, depletion of respiratory substrate is not a cause for the wilting symptom in carnation (Nichols, 1973a).

Using girdling technique and labeled sucrose, Sacalis and Durkin (1972) concluded that exogenous sucrose is taken up initially in the xylem and then translocated to the flower via phloem. However, it has been shown that the phloem does not account for most of the sucrose uptake since girdling only reduces the accumulation of ^{14}C labeled compounds in the petal by 25% (Kaltaler and Steponkus, 1974). Possible means for translocation of exogenous sucrose could be in both the phloem and xylem because lateral movement of sucrose from xylem to phloem always occurs (Chin and Sacalis, 1977a). Pulsing the stem with sucrose solution results in accumulation of reducing sugar in petal tissues and vase life of the flower is nearly doubled (Nichols, 1973a). This finding suggests that applied sucrose is rapidly metabolized and the accumulation of reducing sugar at floral tissues may result from sucrose turnover. Sucrose, after taken up by xylem, is rapidly converted to reducing sugar (Chin and Sacalis, 1977a) suggesting that invertase might be a key factor to control sucrose metabolism in the stem of cut rose. The hydrolysis of sucrose at the receptacle was thought to be a prerequisite for sugar uptake into rose petals (Chin and Sacalis, 1977a), but rose petals can also take up sucrose directly and the hydrolysis of sucrose can occur at rose petals as well (Chin and Sacalis, 1977b).

Exogenous sugar plays an important role in delaying flower senescence. It acts as a chemical agent to maintain cell membrane integrity (Acock and Nichols, 1979; Nichols, 1973b), as an osmoticum (Bravdo *et al.*, 1974; Halevy, 1976), agent causing stomatal closure (Marousky, 1969), or as an agent to maintain mitochondria structure and function (Kaltaler and Steponkus, 1976; Santarius, 1973). Sucrose also interacts with several

growth regulators during cut flower senescence. Flowers treated with sucrose and kinetin showed less response to exogenous ethylene and abscisic acid (ABA) compared to those treated with kinetin alone (Mayak and Dilley, 1976). Sucrose can reduce or nullify the effect of ABA on stomatal closure in rose (Borochov *et al.*, 1976a). The antagonism of sucrose to ABA, stems from the fact that exogenous sucrose lowers the water deficit in the petals, thus reducing the concentration of the ABA (Borochov *et al.*, 1976b).

Starch content: In cut rose, starch level varies with plant tissues (Ho and Nichols, 1977) and variety (Marissen and Brijn, 1995). Starch level in petal and gynoecium of 'Sonia' rose is significantly higher than in leaf and stem (Ho and Nichols, 1977). Starch level in 'Sonia' corolla is 214 $mg \cdot g^{-1}$ DW, whereas that in 'Madelon' is only 76 $mg\ g^{-1}$ DW (Marissen and Brijn, 1995). Starch generally decreases after harvest and is not present in some flowers, such as *Sandersonia aurantiaca* flower, unless the flower is held in a sucrose solution (Eason *et al.*, 1997). During development of Freesia var. 'Aladin', the starch content of the first bud harvested at mature stage is significantly lower than that of the first bud harvested at immature stage and most of the starch in these first buds disappears within 4 days after harvest (Sytsema-Kalkman *et al.*, 1995). The degradation of starch during development of Freesia varies with variety in that the degradation of starch in variety 'Polaris' is significantly faster than that in var. 'Aladin' (Sytsema-Kalkman *et al.*, 1995). Since the degradation of starch is concomitant with the increase in sugar level of rose petal, the rise in sugar level within 3 days after harvest may result from starch breakdown (Ho and Nichols, 1977). On the other hand, starch degradation cannot account for the increase in sugar level during Freesia 'Polaris' development

mainly as it is present in a trace amount (van Meeteren *et al.*, 1995), and there is no starch found in stem of Freesia (Sytsema-Kalkman *et al.*, 1995). Sugar level in rose petals increases after harvest as reported by Ho and Nichols (1977) seem to conflict with other reports, in that the sugar level decreases with time after harvest (Sacalis, 1973). This may be due to the difference in stage of flower development. Ho and Nichols (1977) used young stages of flower development that could bloom further after harvest, hence requiring high level of metabolic substrate. The increase in sugar level after harvest as required by the opening flower is also reported for carnation (Nichols, 1973b), Freesia (van Meeteren *et al.*, 1995) and *Sandersonia aurantiaca* (Eason *et al.*, 1997).

The relationship between starch content in the cut stem and flower vase life is not clear. Rose petal starch level is present at a significant level after harvest and its degradation is concomitant with an increase in sugar level in the same tissue (Ho and Nichols, 1977). Despite the different rates of starch degradation between Freesia 'Aladin' and 'Polaris', the number of floret blooming is not significantly different from each other (Sytsema-Kalkman *et al.*, 1995). Unfortunately, the vase life of flowers in this study was not available. Pulsing the stem with 1.6 % sucrose solution and chasing with 2% sucrose reduces the rate of starch degradation in rose head, leaf and stem after harvest (Sacalis and Chin, 1976). This may be because when other sources of metabolic substrate are available, degradation of starch for metabolic substrate in plant tissue is postponed. Hence, flowers may last longer due to an extra source of energy in the form of starch.

1.3. 6. Plant growth regulators and flower senescence

There is a strong negative correlation between endogenous cytokinins and plant senescence wherein decreased cytokinin levels can induce plant organ senescence (Dumbroff and Walker, 1979; van Staden *et al.*, 1988). Cytokinins change in both quantitative and qualitative ways during growth and development. In young expanding leaves of *Ginkgo biloba*, the major cytokinin found are zeatin and zeatin riboside, whereas in mature and aging leaves zeatin glucoside and zeatin riboside glucoside are the major forms (van Staden, 1976). It was not clear why plants contain so many different cytokinins, but it is proposed that plant tissues metabolize cytokinins in order to regulate a certain form of endogenous cytokinin in a particular organ (van Staden *et al.*, 1988). The stability and extent to which cytokinins are metabolized in a particular organ may determine their effect on senescence (Tao *et al.*, 1983; Zhang *et al.*, 1987).

Application of exogenous cytokinins delay flower and leaf senescence either by increasing water uptake (Mayak *et al.*, 1974; van Meeteren and Gelder, 1980) or delaying chlorophyll and protein breakdown (Goldthwaite, 1972; van Staden *et al.*, 1988). The ability of cytokinin to increase water uptake of the cut flower is thought to correlate with its ability to induce stomatal opening in the dark delaying detached leaf senescence (Thimann, 1979a; Thimann, 1979b; Thimann *et al.*, 1982).

Remobilization of amino acid and nutrients of the cytokinin treated tissues is one mechanism that plant responds to exogenous cytokinins. In cut bean leaves, BA-treated leaves remained green and continued growing whereas, untreated leaves showed senescence symptoms (Leopold and Kawase, 1964). This finding suggests that an

acceleration of the senescence of untreated leaves results from the nutrients levels being drawn down.

In red ginger, dipping inflorescence in 200 ppm of benzyladenine (BA) solution or wax (Folicote) for 10 min increases vase life almost 10 days (Whittaker *et al.*, 1992; Whittaker, 1993). However, the relationship between BA application and the changes in postharvest physiology of red ginger during senescence has not been reported.

1.4. Sucrose metabolic enzymes

Sucrose is the principal product of photosynthesis, a major form of translocated carbon, and a major form of storage sugar in plant tissues (Kruger, 1990). In some species nearly all organs of the plants accumulate high concentrations of sucrose. The level of sucrose in plant tissues depends on three major enzymes that are involved in its synthesis and degradation: sucrose phosphate synthase (UDP-glucose: D-fructose-6-phosphate 2-glucosyltransferase, EC 2.4.1.14), sucrose synthase (UDP-glucose: D-fructose 2 glucosyltransferase, EC 2.4.1.13) and invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26).

The other enzyme involved in sugar metabolism is sucrose phosphatase (sucrose 6-phosphate phosphohydrolase, EC 3.1.3.24) that hydrolyses sucrose-phosphate, a product of sucrose phosphate synthase (SPS) activity, into sucrose. However, due to the large standard free energy change, the reaction catalyzed by sucrose phosphatase is far from equilibrium *in vivo* and when it is coupled with SPS, the entire reaction to produce sucrose is irreversible (Kruger, 1990). Because of its high activity in plant tissues, i.e. 10

fold greater than SPS (Hawker, 1985), the reaction is far from equilibrium *in vivo* and partially inhibited by sucrose, sucrose phosphatase is not considered as one of rate limiting enzymes in sugar metabolism. The reactions catalyzed by these enzymes are summarized in Figure 1.1.

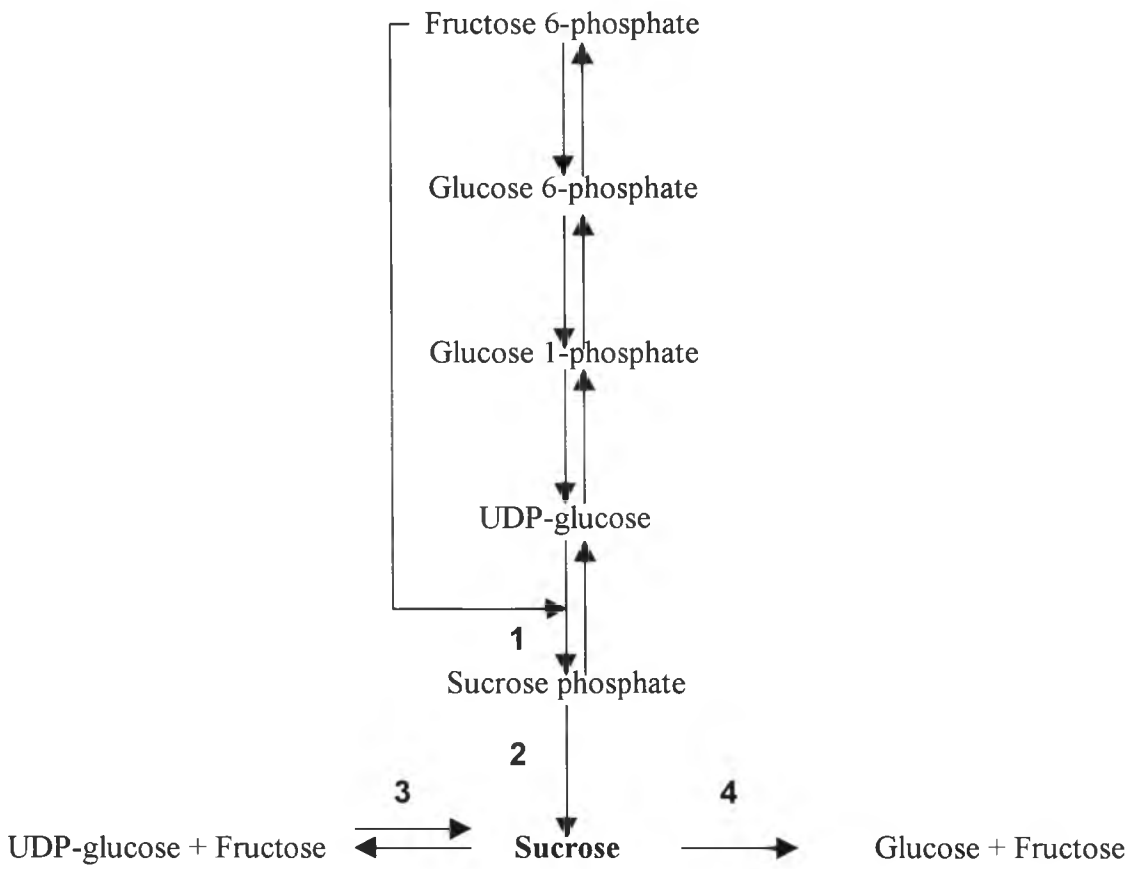


Figure 1.1. Pathways of sucrose metabolism. The numbers denote the following enzymes: 1, sucrose phosphate synthase (SPS); 2, sucrose phosphatase (SP); 3, sucrose synthase (SS); 4, invertase (AI and NI) (modified after Kruger, 1990).

1.4.1. Sucrose phosphate synthase (SPS)

Sucrose is known to be synthesized in cytoplasm from triose phosphates exported from the chloroplasts during photosynthesis through the combined activities of SPS and SP (Huber and Huber, 1992). Although SS can also catalyze the reaction leading to sucrose synthesis, it is not the major route for sucrose synthesis in sucrose producing tissues (Kruger, 1990). SPS activity is usually prominent in chlorophyllous tissues and low in nonphotosynthetic tissue (Avigad, 1982). For example, SPS activity, which is low in young leaves and high in mature green leaves, can be used as one characteristic to determine the transition phase from sink to source tissues (Nguyen-Quoc *et al.*, 1990). Activity of SPS can be regulated either by allosteric effectors or protein phosphorylation. Glucose-6-phosphate serves as allosteric activator, whereas inorganic phosphate is a competitive inhibitor (Crafts-Brandner and Salvucci, 1989; Doehlert and Huber, 1983; Loewe *et al.*, 1996). The phosphorylation of SPS shows diurnal regulation. The phosphorylation of the protein induced by darkness inactivates the enzyme, whereas light induces dephosphorylation, hence reactivates the enzyme activity (Huber and Huber, 1992; Huber *et al.*, 1985). Environmental factors have been reported to affect enzyme activity. During rose development, plants grown under low night temperature (12°C) show relatively low activity of SPS compared to high night temperature (18°C) (Khayat and Zieslin, 1987).

1.4.2. Sucrose synthase (SS)

Sucrose synthase is ubiquitous in higher plants and occurs in most tissues that can metabolize sucrose, especially nonphotosynthetic and storage tissues (Avigad, 1982). However, it is more likely to catalyze the breakdown of the sucrose *in vivo* rather synthesis, particularly when present in growing tissues (Hawker, 1985). In general, the activity of SS is high in actively growing or sugar accumulating tissues and has been used to determine sink strength (Hawker and Hatch, 1965; Nguyen-Quoc *et al.*, 1990; Sung *et al.*, 1989; Wang *et al.*, 1993).

The reaction catalyzed by SS is easily reversible and more likely to be controlled by substrate availability rather than by other types of allosteric modulation (Avigad, 1982). The optimum pH for sucrose cleavage is between pH 6.5 to pH 7.0, whereas for sucrose synthesis, it is around pH 7.5 to pH 8.0 (Hawker, 1985). Activity of SS is found to be positively correlated with tomato fruit relative growth rate and with starch content in the pericarp tissues (Wang *et al.*, 1993). However, in pea epicotyl, acid invertase and SS work separately depending upon locations. In the plumule and hook, the activity of SS is higher than invertase activity, whereas in the expanding region, the activities of these enzymes are *vice versa* (Maclachlan *et al.*, 1970). In general, in youngest leaves, sucrose is cleaved almost exclusively by SS, whereas invertase accounts for about half of the sucrose metabolism in older leaves (Kruger, 1990).

In cut carnation stem, the activity of SS is nearly 10 times higher than invertase activity while in carnation petals, invertase activity is 1.5 times greater than SS (Hawker

et al., 1976). This finding suggests that the possibility of sucrose breakdown by either of these two enzymes varies with plant tissues within a species.

1.4.3. Invertase (AI and NI)

Invertase catalyzes the irreversible hydrolysis of sucrose to glucose and fructose. The forms of invertase found in higher plant are distinguished by having pH optima of about 5.0, for acid invertase (AI), and 7.0, for alkaline or neutral invertase (NI) (Hawker *et al.*, 1976). Acid invertase exists in the vacuoles and is associated with the plant cell wall, whereas alkaline or neutral invertase is probably restricted to the cytoplasm (Dennis and Turpin, 1990). The major forms and hydrolysis functions of the invertase in higher plants vary depending upon plant tissues and species. For example, in the young leaf of citrus (*Citrus sinensis*), the activity of acid invertase is predominant, whereas in the mature leaf, neutral invertase is predominant (Schaffer, 1986). During rose growth and development, the activities of sucrose metabolic enzymes vary with the plant tissues and are regulated by environmental factors. Plants grown under low night temperature (12°C) show relatively low activity of SPS, acid invertase in leaves and SS in young shoot compared to high night temperature (18°C) (Khayat and Zieslin, 1987). In storage tissues, such as sugarcane stem, sucrose hydrolysis by acid invertase in the intercellular space is obligatory for sucrose uptake and subsequently storage for both mature (Hawker and Hatch, 1965) and immature stems (Bowen and Hunter, 1972).

In carnation, Kangaroo Paw, and Sturt pea, acid invertase is predominant in the petal and the activity is 1.5 to 20 fold higher than SS activity (Hawker *et al.*, 1976). This

finding suggests that the degradation of sucrose in flower petals is likely to be catalyzed by invertase activity rather than SS activity.

1.5. Hot water application

1.5.1. Hot water and insect disinfestation

A major problem in the export of tropical cut flower and foliage is insect contamination (Paull, 1991). Suitable insect disinfestation treatments for these commodities are difficult to develop due to wide range of insects and multiple flower morphologies involved. Current disinfestation treatments approved in the United States for cut flowers by the United States Department of Agriculture-Animal Plant Health Inspection Service (APHIS) are limited to hand removal, chemical dips, and methyl bromide fumigation (Hansen and Hara, 1994). Other approaches have also been considered and evaluated such as hydrogen cyanide fumigation (Hansen *et al.*, 1991), vapor heat treatment (Hansen *et al.*, 1992), carbonyl sulfide (Chen and Paull, 1998), and hot water immersion (Hara *et al.*, 1993). However some disadvantages exist in these treatments. Hydrogen cyanide is not a registered fumigant in the United States (Hara *et al.*, 1993); vapor heat treatment requires an expensive facility and highly trained personnel (Hansen and Hara, 1994) and carbonyl sulfide has been recently tested on only one tropical cut flower (red ginger, *Alpinia purpurata*) (Chen and Paull, 1998). Among these treatments, hot water treatment is more promising to eliminate insects in tropical cut flowers and foliage (Hara *et al.*, 1993; Hara *et al.*, 1997; Hara *et al.*, 1996; Tenbrink *et al.*, 1992).

Hot water treatments were developed to eliminate different fruit flies from various tropical fruits such as banana (Armstrong, 1982), mango (Nascimento *et al.*, 1992; Sharp *et al.*, 1988), and papaya (Couey and Hayes, 1986). Recently these hot water treatments were applied to Hawaiian tropical cut flowers (Hara *et al.*, 1993; Hara *et al.*, 1997; Hara *et al.*, 1996; Tenbrink *et al.*, 1992). Temperatures ranging from 47 to 49°C with exposure times varying from 1 to 10 min are used as disinfestation treatments against cockerell scale, *Pseudaulacaspis cockerelli* (Cooley), on bird of paradise leaves (Hara *et al.*, 1993). Hot water at 49°C for 10 min is 100 percent effective in killing all life stages of the cockerell scale on bird of paradise (*Strelitzia reginae* Ait.) leaves, and aphids, ants, and earwigs on red ginger (*Alpinia purpurata*) (Tenbrink *et al.*, 1992). This effective treatment is safe for several foliages such as bird of paradise leaf, anthurium leaf (*Anthurium andraeanum* L.), green ti-leaf (*Cordyline terminalis* L.), a large heliconia (*Heliconia stricta* J. Huber), sago palm (*Cycas revoluta* Thumb), queen sago (*Cycas circinalis* L.) and monstera (*Monstera deliciosa* Leibm.) (Tenbrink *et al.*, 1992). However, the same treatment has been reported to damage calathea (*Calathea insignis* Peters.), lycopodium (*Lycopodium cernuum* L.), raphis palm (*Raphis sp.*) and a small heliconia (*H. psittacorum* L. f.) (Tenbrink *et al.*, 1992).

For red ginger, hot water at 49°C for 12 or 15 min eliminated more than 95% of major insects infesting red gingers such as ants, banana aphids, and mealybugs (Hara *et al.*, 1993; Hara *et al.*, 1996). However, damage from the hot water treatment is a major concern due to bract necrosis and shortened vase life. Damage symptoms from the hot water treatment showed a highly significant correlation with total rainfall in the seven

days before harvest (Hara *et al.*, 1996). Preconditioning red gingers with forced air at 39 ± 1.0 °C for 2 h at air velocity of 0.3 m sec^{-1} and at 61.7 ± 3.3 % RH (Hara *et al.*, 1997) before subjecting them to the hot water treatment induces thermotolerance during winter season when the hot water treatment decreases vase life of the inflorescences. However, conditioning the inflorescence by hot air for 2 h also induces thermotolerance in mealybugs and increases its survival by 0.4 to 40% (Hara *et al.*, 1997).

1.5.2. Effect of hot water on living tissues

When cells are subjected to elevated temperature below the lethal ranges, the synthesis of most normal proteins and mRNAs is repressed, and the transcription and translation of a small set of "heat shock proteins" (HSPs) is initiated (Lindquist and Craig, 1988). This phenomenon is referred to as the heat shock response. These HSPs are produced within 30 min after exposure to temperature in the ranges 34 to 42°C (Kanabus *et al.*, 1984). Among the HSPs induced in response to heat shock, the low-molecular-mass HSPs (15 to 25 kilodaltons) are the most abundant and unique in plants (Mansfield and Key, 1987). These proteins are reported to correlate with thermotolerance characteristics (Chou *et al.*, 1989; Yeh *et al.*, 1994). A relationship between heat shock proteins and thermotolerance have been reported for cultured plant cells (Wu and Wallner, 1984), sorghum (Ougham and Stoddart, 1986), soybean seedling (Lin *et al.*, 1984), and field-grown papaya fruit (Paull and Chen, 1990). The decay of HSPs occurs within 24 h, with a corresponding loss in thermotolerance (Paull and McDonald, 1994).

1.5.3. Physiological effect of heat treatment

Heat treatment, alone or combined with other postharvest treatment, has been used extensively as a treatment to maintain postharvest quality prior to long term storage of several fruits and vegetables (Paull and McDonald, 1994). Physiological effects of heat treatment vary with plant species. For example, heat treatment (38°C for 4 days) reduces superficial scald of apple (Lurie *et al.*, 1990) and hot air (from 47 to 50°C for 2 to 4 h) reduces microbial spoilage during storage (at 21°C) of onions (Thamizharasi and Narasimham, 1993).

Heat treatment reduces chilling injury (CI) in several fruits and vegetables. For example, a hot water dip at 53°C for 2 to 3 min reduces significantly the sensitivity of citrus fruit when stored at 2°C for 4 to 8 weeks (Rodov *et al.*, 1995). For zucchini squash, hot water at 42°C for 30 min reduces CI of the squash stored at 5°C (Wang, 1994), whereas hot air at 38°C for 8 to 12 h is recommended for reducing CI in avocado (Nishijima *et al.*, 1995). When combined with fungicide (thiabendazole, 4 g a.i./L), hot air (48.5°C for 3 to 4 h) significantly reduces postharvest diseases in papaya (Nishijima *et al.*, 1992).

Prestoring apple for 4 days at 38°C before storage at 0°C inhibits the development of superficial scald for up to 5 months (Lurie *et al.*, 1990). This phenomenon may due to heat treatment inhibiting the accumulation of α-farnasene and conjugated trienes in the apple cuticle (Lurie *et al.*, 1990). Heating apple before cold storage also induces changes in membrane components in which the ratio of sterol/phospholipid increases after heat,

but decreases during cold storage (Lurie *et al.*, 1995). Lurie *et. all* (1995) conclude that treated fruit benefit the first stress (heat treatment) by acclimating more successfully to the second stress conditions (cold storage) than do untreated fruit.

Effective heat treatment used to prevent CI vary with commodities, such as 53°C for 2 to 3 min for citrus fruits (Rodov *et al.*, 1995), 42°C for 30 min for zucchini squash (Wang, 1994), and 38°C for 8 to 12 h for avocado (Nishijima *et al.*, 1995). A major effect of heat treatment is on ethylene production. In general, ethylene production exhibits a Q_{10} value of about 2, between 20 to 40°C with further increases in temperature generally resulting in a decline in the rate of ethylene production (Field, 1985; Saltveit and Dilley, 1978). The effect of temperature on ethylene production varies with plant species and incubation time. For example, ethylene production is decreased if the apple tissue is incubated (12 h) at temperature higher than 30°C (Yu *et al.*, 1980), whereas holding mature green tomato at temperature higher than 27°C increases ethylene production (Biggs *et al.*, 1988). It has been suggested that the inhibition of ethylene production by high temperature up to 35°C does not appear to be associated with permanent tissue damage, since return of the fruit to a lower temperature results in the resumption of ethylene production (Biggs *et al.*, 1988).

Heat inactivation of ethylene production could have its effect either on ACC synthase resulting in the depletion of ACC content (Biggs *et al.*, 1988) or on ACC oxidase which results in the accumulation of ACC content (Dunlap *et al.*, 1990; Yu *et al.*, 1980). It has been suggested that the initial phase of ethylene production at high temperature is due to the repair of the losses in ACC synthase activity, whereas the

second phase is due to a reduction in ACC oxidase activity (Biggs *et al.*, 1988). Since heat treatment causes several changes in membrane integrity (Lurie *et al.*, 1995), the inactivation of ethylene production by heat treatment might result from the inhibition of membrane-associated ACC oxidase as reported for apple tissue (Yu *et al.*, 1980), muskmelon (Dunlap *et al.*, 1990), papaya (Chan, 1986a) and cucumber (Chan, 1986b).

Besides its effect on ethylene production, hot water effects have been reported for respiration rate of some commodities. Cucumber submerged in hot water (25, 38, and 42°C) for 30 min shows a decrease in respiration when the respiration rate is determined 48 h after the hot water treatments when compared to the original determination (0 h). The greatest reduction is found at the highest temperature, 42°C (McCollum *et al.*, 1995). Concomitant with a lower respiration rate, hot water treated cucumber shows a reduction in ethylene production and ACC oxidase activity, whereas ACC content is slightly higher than that of the control. This finding confirms the effect of heat treatment on ACC oxidase activity in cucumber (Chan, 1986b).

The physiological effect of hot water on postharvest physiology of cut flower has not been documented although it has been reported to extend postharvest vase life of some cut flowers such as red ginger (Hara *et al.*, 1997; Hara *et al.*, 1996) and *Papaver nudicaule* L. (Farina *et al.*, 1989).

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1. Plant materials and general practice

Red ginger inflorescences were normally harvested (70-75 cm) from plants grown at Poamoho Experiment Station on the Island of Oahu. Inflorescences were harvested at the two-thirds open stage in the morning and transported in water to the laboratory within 3 h. The two-thirds open stage referred to when two-thirds of the inflorescence bracts were open from the rachis. This stage is commonly known as the commercial stage. Standard-size inflorescences (20 cm long) were selected and damaged inflorescences culled before installation of the experiments.

For vase life evaluation experiments, stems having one leaf were cut to the same length (55 cm) and washed with 7.5 $g \cdot l^{-1}$ Liqui-Nox detergent (Alconox Inc., N. Y.) to remove aphids and other insects before being left to dry overnight while standing in tap water. In other tests, stems without the leaf were cut to 40 cm before washing and the leaf sheath then removed and allowed to dry overnight, while standing in water. On the following day, stems were recut under water to have the same stem length (35 cm) before being used as starting materials for physiological studies.

Tap water was routinely used during preparation for various experiments unless otherwise indicated. The mineral composition of the tap water is shown in Appendix G. All other solutions in the studies were prepared by sing deionized (DI) water.

For vase life evaluation, simulated shipping was conducted after the inflorescences were dried overnight. The inflorescences were packed horizontally in cardboard carton, lined with plastic (20 μm thickness) and packed with moistened shredded newspaper. Cartons were kept at room temperature (22 ± 2°C) for two days before unpacking. After unpacking, 5 cm of the basal end was removed under water before the inflorescences were placed in plastic buckets containing 3 L of tap water. Evaluation of vase life was done every other day on inflorescences held at 22 ± 2°C, 60 – 70% relative humidity (RH), and 12 h per day of fluorescent light (15 ± 4 $mmol \cdot M^{2} \cdot s^{-1}$).

2.2. Evaluation of vase life

The end of vase life was determined for inflorescence wilting or drooping symptom (Fig. 2.1) and browning of the inflorescence due to the discoloration of the distal bracts or center bract browning symptom (Fig. 2.2). Scores for senescence symptoms were rated on a scale of 0 to 4 for each symptom (Table 2.1). Inflorescences were regarded as unmarketable when the senescence score was 1 or 2 and the vase life ended when the score was 3 or 4. All inflorescences were evaluated individually.

2.3. Determination of postharvest physiological factors

2.3.1. Ethylene production and respiration rate

Six inflorescences without the leaf sheath (stem length = 35 cm) were weighed and placed in a 300 ml beaker filled with 150 ml deionized (DI) water before being placed in the plastic chamber (10.2 L) sealed with the plastic lid. There were three

chambers for each treatment. After 1 h, triplicate sample of 1 ml of headspace sample were removed and injected into a nitrogen gas flow leading to a carbon dioxide analyzer (LI 6251, LI-COR Inc., Lincoln, Nebraska) for determination of CO_2 production (Paull *et al.*, 1985). An additional, triplicate of 1 ml of headspace sample were withdrawn after 6 h and injected into a gas chromatograph (GC) (GC-8A, Shimadzu Corporation, Kyoto, Japan) for determination of ethylene production (Paull *et al.*, 1985). Samples were separated using alumina column (5' x 1/8', 60/80 mesh) and ethylene was determined by a photoionization detector (PID). The GC was equipped with a digital integrator (CR501 Chromatopac, Shimadzu Corporation, Kyoto, Japan). Injector port, column, and the detector port were held at 70°C, 70°C and 120°C, respectively. Helium carrier gas was supplied at a flow rate of 30 $ml \cdot min^{-1}$. After the samples were taken, the chambers were opened and aerated, then kept at room temperature (22 ± 2°C, 50 to 60 % RH) till the next measurement. Data were taken every other day until the end of vase life.

2.3.2. Water balance of the cut stem

For water uptake experiments, 35-cm stem length of standard size inflorescences without leaves and leaf sheaths were used. Ten individual inflorescences were used per treatment and each inflorescence was determined separately (n = 10). The area of the cut surface was calculated for an elliptical area: area = (L)(W)* 0.8 (Hensley, 1994) where L= length and W = width of the cut surface. Inflorescence fresh weight (FW) was determined, and the change in FW over time was expressed as percentage of the original weight (% FW). After determining for initial fresh weight, each inflorescence was placed

in a 50-ml test tube filled with DI water or holding solution that was maintained at the same level (50 ml) until the experiment ended. Evaporation of water from the containers was prevented by adding 1 ml of paraffin oil to each test tube (O.D. 25 mm). The paraffin oil was found to be inert to flower stems such as the gladioli shoots (Bravdo *et al.*, 1974). Thus daily loss in the whole system weight was considered as transpiration (water loss) while the amount of water or solution added to bring the level of solution to the same level (50 ml) was considered as water uptake. The differences between consecutive weighings (test tube with stem) after adding water was used to calculate fresh weight loss per day. Data were taken daily until end of inflorescence vase life.

2.3.3. Stem and vase microorganisms

Microorganisms on the stem segments and in the vase solution were determined using methods developed for carnation (Mayak *et al.*, 1977; van Doorn *et al.*, 1995b) and rose (Hoogerwerf *et al.*, 1989; van Doorn *et al.*, 1989). Inflorescences were prepared as previously described in section 2.3.2 for setting up the water balance experiment, except without the 1 ml of paraffin oil. Water in the test tube was maintained at the same level (50 ml) until the vase life ended. At a specific date after experiment was set up, stems (5 stems/treatment/sampling date) were removed from vase solution in a lamina-flow chamber and 80% ethanol used to disinfect the outside stem. A segment (ca. 2-cm long) was cut from the cut end, weighed for fresh weight, and placed in the sterile plastic bag (10.2 cm x 15.2 cm, Stomacher, Seaward Medical). Five ml of autoclaved DI water was added and the bag carefully sealed without air bubbles. A rubber mallet was used to

smash the stem segment for 30 seconds. One-ml aliquot of the solution was used for serial dilution. Duplicates of the 100 µl diluted solution were spread separately on a sterile plate count agar (PCA) (Difco, Laboratories, Detroit, MI). Cultures were incubated at 37°C for 3 days before determining the total number of microorganisms, expressed as the number of colony forming units per gram fresh weight of stem section ($cfu \cdot g^{-1}$ FW). The number of bacteria in the vase solution was determined by spreading 100 µl diluted vase solution on a sterile PCA plate and incubated as previously described. The number of total microorganisms in the vase solution was expressed as the number of colony forming units per milliliter of vase solution ($cfu \cdot ml^{-1}$).

2.3.4. Total sugar, sucrose and reducing sugar

Extraction of sugar from fresh tissues: Triplicate 2 g sample of finely chopped tissues were heated in microwave oven for 1.3 min to inactivate the enzyme activities and homogenized (Ultra Turrax homogenizer, Texmar Company, Cincinnati, OH) with 18 ml of 90% (v/v) ethanol. The homogenate was allowed to stand for at least 2 wks at 4°C to clear the supernatant. The protocol to extract sugar from fresh tissue is summarized in Appendix A.

Extraction of sugar from frozen tissues: Frozen tissues were ground to a powder in the presence of liquid nitrogen. In triplicate, deionized water (1.7 ml) was added to each of 2 g ground tissues, and the tubes were heated to inactivate enzyme activities for 1.3 min in a microwave oven at full power (EM 3620, Sanyo Electric Inc., Little Ferry, N. J.). The tissues were homogenized for 15 seconds with 18 ml of 90% (v/v) ethanol.

After homogenizing, the homogenate was centrifuged at 11,672 x g (10,000 rpm, SA 600 rotor, Sorvall RC-5B centrifuge, Du Pont Instruments, Newtown, CT) at 4°C for 20 minutes. Clear supernatant was transferred to capped bottle and kept at 4°C until used. The protocol to extract sugar from frozen tissue is summarized in Appendix B.

Sugar determination: Total ethanol-soluble sugars was determined by the phenol-sulfuric acid procedure (Dubois *et al.*, 1956; Robyt and White, 1987) in which 100 µl of 5% aqueous phenol was added to 100 µl sample solution. The solution was mixed and 1.0 ml of concentrated sulfuric acid added. After the mixture had been allowed to stand for color development at room temperature (20 min), the absorbance was read at A_{490} nm using a spectrophotometer (UV 160U, Shimadzu Corporation, Kyoto, Japan). The level of total ethanol soluble sugar was quantified by comparison to a standard curve using known amount of glucose, representing as glucose equivalent unit per mass unit.

Sucrose was determined after reducing sugar was destroyed at high temperature in the presence of strong base (van Handel, 1968). In this procedure, 100 µl of 30% (w/v) KOH was added to 100 µl sample solution and the mixture held in a boiling water bath for 10 min. After cooling in tap water for 10 min, sugars in the sample were determined using phenol-sulfuric acid method as previously described (Dubois *et al.*, 1956). Concentration of sucrose was quantified by comparison to a standard curve using known amount of sucrose, representing sucrose equivalent unit per mass unit. Indirectly, the level of reducing sugars can be determined by the difference between the determination of total sugars with and without the presence of 30% KOH.

Reducing sugar was determined directly by 1% of 2-cyanoacetamide in the presence of 100 mM borate buffer, pH 9.0 (Honda *et al.*, 1982). In this procedure, 100 µl of 1% 2-cyanoacetamide was added to the 100 µl sample solution. One ml of 100 mM borate buffer was added and mixture boiled in a water bath for 10 min. After being cooled in tap water for 10 min, absorbance was read at A_{276} nm using spectrophotometer (UV 160U, Shimadzu Corporation, Kyoto, Japan). The level of reducing sugar was quantified by comparison to a standard curve using known amounts of glucose and fructose as standard. The protocols to determine sugar contents are summarized in Appendix C.

2.3.5. Starch level

After sugars were extracted from the tissues, the residue after centrifugation was used for starch determination. The residue was washed twice with 18 ml of 90% ethanol to remove soluble sugars and pigments. After each wash, the mixture was allowed to stand in cold room (4°C) to clear the solution before the next wash was performed. After the final wash, the residue was placed in the oven (55°C) to dryness for 14 days. In triplicate, 150 mg of dry sample was placed in a screw cap test tube. Five ml of DI water was added. Nitrogen gas was blown into the tube for about 1 minute before the tube was quickly capped. Starch was solubilized by boiling in a water bath for 1 h and the mixture left for 24 h at room temperature with the cap securely closed. Five ml of 1.0M KOH was added to each tube. After mixing, the mixture was allowed to stand to clear the solution at room temperature for 7 days. A 0.5 ml aliquot of the cleared solution, 0.5 ml

of 0.5 M HCl was used to neutralize the sample. A 10 mg potassium bi-tartrate was added to the tube and the volume was brought to 2.4 ml with DI water. A 100 µl IKI (0.2 g of iodine (I_2) plus 2.0 g potassium iodide (KI), in 100 ml) solution was added and mixed. The mixture was allowed to stand for 20 min at room temperature (22 ± 1°C) for color development before the absorbance was read at A_{680} nm using spectrophotometer (UV 160U, Shimadzu Corporation, Kyoto, Japan). The concentration of starch was quantified by comparison to a standard curve using known amount of soluble starch (potato).

2.4. Data analysis

Experiments were conducted at least twice and the data analyzed by either or both the General Linear Model Procedure (GLM) or analysis of variance (ANOVA) procedure. Where possible, mean comparisons were made using Waller-Duncan multiple range test. Statistical tests were carried out using the SAS system (SAS Institute Inc., Cary, N.C.).

Table 2.1 Criteria used to evaluate vase life of red ginger inflorescence.

Scale	Criteria
0	No senescence symptom development
1	Center of bracts discolor, ≤ 5% of total bracts always browning, or tip starting to bend away from the axis
2	5 – 10% of total bracts brown, or tip bent away from the axis ≤ 30°
3	20 – 30% of total bracts brown, or tip bent away from the axis ≤ 90°
4	30 – 50% of total bracts brown, or tip bent away from the axis ≥ 90°

Figure 2.1. Rating scale for inflorescence wilting or drooping symptom of red ginger inflorescence (Table 2.1).

Figure 2.2. Rating scale for center bract browning symptom of red ginger inflorescence (Table 2.1).

CHAPTER 3

POSTHARVEST PHYSIOLOGY OF RED GINGER SENESCENCE

3.1. Introduction

Red ginger (*Alpinia purpurata* Vieill. K. Schum) is commonly used as an ornamental. But, as with other tropical cut flowers, information on postharvest physiology and postharvest factors affecting flower vase life is limited. Vascular blockage of cut stem leads to the bent neck condition in cut rose (Durkin and Kuc, 1966), water loss from flower significantly precedes stem breakage in gerbera (van Meeteren, 1978a) and rolling and discoloration of petal correlates with ethylene production (Gilissen, 1977). However, none of these types of relationships have been described for red ginger inflorescence.

Senescence in red ginger inflorescence is described as inrolling of the leaves and darkening of inflorescence bracts (Paull, 1991), but no attempts have been made to understand the relationship between these senescence symptoms and postharvest physiological factors. Several postharvest factors have been suggested to affect postharvest vase life of red ginger such as the attached leaf on the stem (Akamine, 1976; Rietow, 1986), and stem length (Broschat and Donselman, 1988), but these reports provided no tables or figures to illustrate the data.

The postharvest factors involved in cut flower senescence vary greatly with commodity, due to genetic background, morphology and physiology of the cut flowers. While ethylene has a significant effect on the postharvest vase life of carnation (Jones

and Woodson, 1997), and it has little effect on postharvest vase life of gerbera (van Meeteren, 1978b).

Red ginger inflorescence is composed of colored bracts or modified leaves attached to the main rachis (Dahlgren *et al.*, 1985). Hence, the senescence symptoms and their development may be different from true flowers such as rose or carnation. The objectives for this study, therefore, are to determine the postharvest factors associated with red ginger senescence and the relationship between senescence symptoms and postharvest physiological factors and provide a possible explanation for these senescence symptoms.

3.2. Materials and methods

3.2.1. Senescence symptoms

Inflorescences used for senescence observation were harvested monthly from Poamoho Experiment Station for up to 4 months. After being packed and unpacked, inflorescences were placed in the buckets containing tap water for vase life evaluation. Individual inflorescence was evaluated for vase life, and the major symptoms causing a loss of vase life were observed. Senescence symptoms of red ginger were determined as previously described in general materials and methods (Table 2.1, Fig. 2.1 and 2.2). For each senescence symptom, the number of inflorescences affected was recorded, and data pooled for analysis.

3.2.2. Vase life and inflorescence characteristics

Inflorescence maturity: Inflorescences were harvested at different stages of development and postharvest vase life was determined. The four different stages of development were: bud stage, 1/3 open, 2/3 open, and full open (Fig. 3.1), designated as stage 1, 2, 3 and 4, respectively. The bud stage referred to the stage at which the elongated inflorescence has no open bracts. This stage corresponded to the development of the inflorescence at 3 weeks after the inflorescence emerged from the leaf sheath (W-3, Fig. 3.1). The development stages of the bract opening following the bud stage were one week apart from each other. For example, the open stage of inflorescence bracts was defined as 1/3 open when bracts at the top of the inflorescence (1/3 of the total bracts) start to open, and this was at week 4 of the development. After simulated shipping, postharvest vase life of the inflorescences was evaluated. The experiment was performed twice and data pooled for analysis.

Stem length: Stem length of the inflorescences (measured from the base of the inflorescence to the cut end) was varied 30, 50 or 70 cm before packing and the postharvest vase life of the inflorescence was determined using the senescence scores (Table 2.1). Inflorescences were harvested monthly for a total of 12 months and data pooled for statistical analysis.

Stem diameter: After washing, inflorescences were classified into two groups: standard size (diameter at the middle of the stem $\geq$ 10.0 mm) and small (diameter at the middle of the stem $\leq$ 10.0 mm). Postharvest vase life of the inflorescences was observed

after simulated packing. The experiment was performed twice and one experiment was selected to represent the results.

Leaves attached to the stem: Inflorescences were harvested and the leaf left attached or removed. After shipping simulation for 2 days, the postharvest vase life was determined. The experiment was performed twice and data pooled for analysis

3.2.3. Effect of postharvest handling

Packing methods: Four different packing methods were evaluated: 1, packed dry, without a plastic liner in the carton; 2, packed dry, with a plastic liner in the carton; 3, packed with moistened, shredded newspaper, without a plastic liner in the carton; and 4, packed with moistened, shredded newspaper, with a plastic liner in the carton. The plastic liner was a 20 μm thick polyethylene sheet. For this study, inflorescences not packed were used as the control. Experiment was conducted with both standard and small size inflorescences and with and without the presence of one leaf attached to the stem. The experiment was performed twice and data pooled for statistical analysis.

Holding solutions: Inflorescence were prepared as for the physiological study where the leaves and leaf sheaths were removed and stems recut to a final length of 35 cm after simulation of 2 days shipment. Six different holding solutions were tested: deionized water (DI), 2% (w/v) sucrose (pH 5.6), 200 ppm HQC (pH 4.2), 1 mM citric acid (pH 3.4), 2% (w/v) Florever (Floralife Inc., Walterboro, SC, pH 3.1), and 2% (w/v) sucrose plus 200 ppm HQC (pH 4.2). There were 10 inflorescences per treatment. Solution uptake and loss rate of individual stem was determined daily for up to 7 days.

Vase life was determined after the data was collected. The experiment was performed twice and one experiment was selected to represent the results.

Ethylene inhibitors: Ethylene inhibitors were used to determine indirectly the effect of ethylene on inflorescence vase life. Four different postharvest treatments were tested: 1, control, no application of ethylene inhibitor; 2, pulsed 6 h with 1:4 STS (1 mM silver nitrate: 4 mM sodium thiosulfate) before packing; 3, gassed for 6 h with 500 ppb 1-methylcyclopropene (MCP) before packing; and 4, vase solution with 25 μM amino-oxy acetic acid (AOA) after unpacking. Most inflorescences were held in tap water for vase life evaluation, except the AOA treatment that 25 μM AOA was used. The experiment was performed twice and data pooled for statistical analysis.

Benzyladenine (BA) application: Inflorescences, with or without one leaf attached to the stem were thoroughly sprayed with BA (200 ppm). The treatment was applied after washing and left to dry overnight before being packed for 2 days. After unpacking, inflorescences were evaluated for vase life every other day until end of vase life. The experiment was performed twice and data pooled for analysis.

3.2.4. Postharvest physiology of red ginger senescence

Inflorescences without leaf sheaths (35 cm stem length) were used throughout this study. Ethylene production, respiration rate, water balance of the cut stem, stem and vase microorganisms, total sugar, sucrose and starch level were determined after harvest. Results were compared between untreated and BA-treated inflorescences. Determination of ethylene production, respiration rate, water uptake and water loss rate was performed

daily until end of vase life. Experimental set up was as previously described (Section 2.3.1 for ethylene and carbon-dioxide production, and section 2.3.2 for water uptake and water loss rate experiment). Experimental set up for total count microorganisms and extraction of starch and sugars was described previously (Section 2.3.3 and 2.3.4, respectively) with some modification. Samples for total count microorganisms, and extraction of starch and sugar were taken on 1, 3, 6, 9, 12, 15, 18 and 21 days after the treatment or until end of vase life. For each sampling date and treatment, samples were combined from five inflorescences. Stem at the cut end (2-3 cm) was used to determine total microorganisms in individual stem section. Bracts from the apical end of the inflorescences were combined for the bract sample, whereas the middle section of the stem (15 cm long) was combined to represent the whole stem. The extraction of starch and sugars for each sample was conducted three times. The protocols used to extract and determine sugar content are summarized in Appendix A, B, and C. All experiments were performed twice and one experiment selected to represent the results.

3.3. Results and discussion

3.3.1. Senescence symptoms

Senescence symptoms in red ginger were classified into 3 types (Table 3.1). The first symptom was inflorescence wilting or drooping symptom (DP) due to the bending of the inflorescence away from the main axis and the inrolling of the leaves. This symptom seems to correlate with vascular blockage of the cut stem as mentioned earlier (Paull, 1991). Wilting symptoms of any kind in cut flowers is an indicator of vascular tissue

blockage caused either by a physiological process, air embolism, or the growth of microorganisms (Halevy, 1976). The second symptom was center bract browning (CB) due to the discoloration of the young bracts at the distal end, later they turned brown and dried out at the end of vase life. The last symptom was bract darkening (DK) when the whole inflorescence turned dark red without wilting or browning of the distal bracts.

The number of inflorescence observed for each senescence symptom varied with harvest date during the 4-month study period (Table 3.1). In February and March, more than 50% of inflorescences were terminated because of inflorescence drooping, whereas for the last two months, 75 to 80% of inflorescences were terminated by center bract browning. Throughout this study, bract darkening contributed fewer than 10% of the total inflorescences. Hence, the evaluation of vase life for all subsequent experiments was based on only the first two senescence symptoms (Table 2.1). The presence of bract darkening in the subsequent studies, if any, was considered as an outlier and excluded from the results for statistics analysis.

3.3.2. Vase life and inflorescence characteristics

Inflorescence maturity: Stage of inflorescence development was responsible for variation in vase life of the red ginger (Fig. 3.2). The younger the stage at which the inflorescences were harvested, the shorter the vase life (R^2 = 0.97, n = 80). Bud inflorescences wilted and failed to open even when held in sucrose solution (Table 3.2). This finding suggested that red ginger cannot be harvested at the bud stage and forced to open in the sucrose solution as successfully reported for other commercial cut flowers

such as gladiolus (Halevy and Mayak, 1974) and chrysanthemum (Marousky, 1973). However, the variation in inflorescence vase life also increased if the inflorescences were harvested at more mature open stages.

Stem length: Previous studies on the effect of stem length showed that the longer the stem length, the longer the inflorescence vase life (Broschat and Donselman, 1988). However, we found that this effect was true only when the inflorescences were not packed when the vase life of long stem (70 cm) increased 1.5 folds over the short stem (30 cm) (Table 3.3). After being packed for 2 days, there was no significant effect of stem length on inflorescence vase life (ranged 18.3 to 20.5 days, Table 3.3). Analysis of variance of the pooled data, showed the significant effect of both stem length (P = 0.0001) and packing (P = 0.0001), on inflorescence vase life. In addition, there was a significant interaction between the two factors (P = 0.01). Packing was not studied by Broschat and Dolselman (1988). Packing reduced vase life 20% over non-packed inflorescences. These results for inflorescences subjected to packing are more meaningful as the inflorescences are normally packed for shipment.

Stem diameter: It has been reported that inflorescences with small diameter stem have shorter vase life i.e., 5 days (Broschat and Donselman, 1988). However, neither stem diameter nor inflorescence size had any significant effect on red ginger vase life (Table 3.4). This effect differed from Broschat and Donselman (1988) and may result from the negative influence of pacing on vase life as found for stem length (Table 3.3). This finding suggested that factor other than stem diameter or inflorescence size affects red ginger vase life.

Leaf attached to the stem: It was suggested that all leaves should be removed before packing, otherwise the vase life of the red ginger would be reduced (Akamine, 1976; Rietow, 1986). In our study, the presence of one leaf attached to the stem had no effect on postharvest vase life of the red ginger (Table 3.5), regardless of the inflorescence size. The high variation found in this study may result from the fast development of wilting symptom compared to the slow development of center bract browning. Postharvest vase life of some cut flower, such as chrysanthemums, protea or statice depends on the foliage to be an energy resource (Dai and Paull, 1995; Halevy and Mayak, 1979). In roses and carnations, removal of the leaves reduces both the rate of water uptake and water loss rate from the cut stem (Carpenter and Rasmussen, 1974), hence having a significant effect on postharvest vase life of these cut flowers. However, this finding cannot be generalized to red ginger as the cut stem of red ginger (50 cm) had only 2 to 3 nodes to where leaf sheaths and leaves can be attached. Hence, the interaction between leaves and the inflorescence in red ginger may not be significant for vase life. In addition, the leaves and the inflorescence develop senescence symptoms independently (Tjia, 1988).

3.3.3. Effect of postharvest handling

Packing methods: As previously reported, packing can nullify the effect of stem length on vase life of red ginger (Table 3.3). However, the choice of packing materials and methods vary depending upon the availability of materials and local practice. The packing experiments were conducted to simulate packing systems used in Hawaii. The

polyethylene liner inside the cardboard box had a significant effect on postharvest vase life of red ginger (P = 0.03), whereas moistened newspaper had no effect on the inflorescence vase life (Table 3.6). The results suggested that, whether the inflorescences were packed wet (with moistened, shredded newspaper) or dry (shredded newspaper not moistened), the plastic liner inside the box cannot be omitted.

Holding solutions: Broschat and Donselman (1988) reported that postharvest vase life of red ginger increased from 10.3 days to 15.0 days when compared the stems held in DI water and holding solutions containing 2% sucrose and 200 ppm hydroxyquinoline citrate (HQC). However, information on solution uptake and changes in inflorescence fresh weight of the cut stem was not reported. In this study, stems without leaf sheath were used, since leaves and leaf sheaths had previously been shown not to contribute to inflorescence vase life (Table 3.5). Fresh weight of the inflorescences held in solutions containing sucrose (2% sucrose, 2% Florever, and 2% sucrose plus 200 ppm HQC) increased during the 7 days of monitoring (100.3%), whereas those held in the solutions without sucrose (DI water, 200 ppm HQC, and 1 mM citric acid) decreased (99.9%) (P = 0.0001, Fig. 3.3A). However, the rates of solution uptake for the stems held in sucrose-containing solutions were lower (0.09 ml/flower/day) than those held in solutions without sucrose (0.11 ml/flower/day) (P = 0.006). The vase life of the inflorescences from sucrose-holding solution was significantly longer (23 days) than those from holding solutions without sucrose (13 days) (P = 0.0001, Fig. 3.3B). Water loss rate from the inflorescences held in sucrose-containing solution was lower (0.09

ml/flower/day) than those held in solutions without sucrose (0.10 ml/flower/day) (P = 0.003).

Sucrose in the holding solution frequently extends cut flower vase life (Halevy and Mayak, 1981). Sucrose in holding solution, once taken up by the cut stem, can act as respiratory substrate (Coorts, 1973), maintaining mitochondrial structure and function (Kaltaler and Steponkus, 1976), and improve water balance and osmotic potential of cut flower (Acock and Nichols, 1979). In this study, holding red ginger in sucrose-containing solutions increased inflorescence fresh weight, at least for the first 7 days after harvest, and extended inflorescence vase life.

Quinoline salts i.e., hydroxyquinoline citrate (HQC) are known to be fungistatic, controlling microorganism growth in holding solution (Larsen and Cromarty, 1967) and act as acidifying agent to improve water uptake of the cut stem (Marousky, 1971). These salts are normally present in most commercial preservative solutions (Halevy and Mayak, 1981). In red ginger, quinoline salt, alone, failed to extend inflorescence vase life (Fig. 3.3B). This finding implied that the growth of microorganisms in holding solution had little effect on postharvest vase life of red ginger. However, this experiment was not conducted to determine the number of microorganisms in the stem segments and holding solutions.

Holding solutions having low pH have been shown to enhance cut flower vase life by increasing water conductivity of the cut stem (Durkin, 1979). However, results showed that low-pH solutions (1 mM citric acid, pH 3.4, and 2% Florever, pH 3.1) did not have a significant effect on enhancing water uptake of the cut stem over the control

(DI water, pH 5.8). Low-pH solution alone (1 mM citric acid) did not significant increase inflorescence vase life compared to the control, whereas low-pH solution containing sucrose (2% Florever) increased vase life 1.7 fold over the control (P = 0.0001) (Fig. 3.3B). This finding suggested that rate of solution uptake, alone, cannot be used as an indicator to predict vase life.

Ethylene inhibitors: Ethylene enhances senescence in several flowers (Mayak and Tirosh, 1993; Nichols, 1968; Paull and Goo, 1985; Reid, 1989; Reuveny *et al.*, 1993). However, the effect of ethylene on red ginger vase life has not been reported. Effect of ethylene on postharvest vase life was implied indirectly from the application of ethylene inhibitors. Amino-oxy acetic acid (AOA) is an inhibitor of ACC synthase, a key enzyme in ethylene biosynthesis (Abeles *et al.*, 1992). Silver thiosulfate (STS) is a competitive inhibitor of ethylene action (Veen, 1986) and 1-methylcyclopropene (MCP) is a gaseous binding-site competitor to ethylene (Serek *et al.*, 1995), hence preventing damage from both internal and external ethylene. None of these ethylene inhibitors extended red ginger vase life (Fig. 3.4). Indeed, STS caused tissue damage at the cut end (tissues turned black), wilting of the inflorescence, and leaves. This finding suggested that vase life of red ginger was not greatly impacted by ethylene. This was not an unexpected result as red ginger bracts are modified leaves (Dahlgren *et al.*, 1985) that, in general, produce less ethylene than do floral parts when they senesce (Brandt and Woodson, 1992; Franco and Han, 1997).

Benzyladenine (BA) application: BA extends postharvest vase life of several commodities, such as broccoli (Downs *et al.*, 1997), leaf (Smart *et al.*, 1991), and several

cut flowers (Lukaszewska *et al.*, 1994; Mor *et al.*, 1983; van Meeteren and Gelder, 1980; Whittaker *et al.*, 1992). It has been proposed that cytokinins prevent the increase in proteolytic enzyme activities during senescence (Mok, 1994), hence, preventing membrane breakdown and delaying leaf senescence. The effect of BA on postharvest vase life of red ginger has already been reported, included with other tropical cut flower such as anthurium (*Anthurium andraeanum*) and heliconia (*Heliconia psittacorum*) (Whittaker, 1993). The results from this study confirmed that BA extended postharvest vase life of red ginger inflorescences and leaves (Table 3.7). Therefore, subsequent experiments on postharvest physiology of red ginger were conducted using 200 ppm of BA. These results also confirmed the previous finding that attached leaf did not have any effect on postharvest vase life of red ginger and that vase life of inflorescence bracts and leaves were independent (Table 3.6). However, vase life of control leaf in this study was longer than that from the previous study. This might result from the variation in plant materials in which the previous study (Table 3.6) was conducted in April 1995, whereas this study (Table 3.7) was conducted in March 1996. In addition, it has been reported that environmental factors have some effect on postharvest vase life of red ginger (Hara *et al.*, 1996).

3.3.4. Postharvest physiology of red ginger senescence

Changes in ethylene production, respiration rate, stem and vase solution microorganisms, sugar and starch contents were compared between untreated control and BA (200 ppm) treated inflorescences.

Ethylene production and respiration rate: Postharvest production of ethylene in red ginger was very low, compared to the respiration rate. For both groups of inflorescences, ethylene production increased slightly with time after harvest until the inflorescence senesced (Fig. 3.5A). The respiration rate of the inflorescences initially declined with time (Fig. 3.5B), however, once the control inflorescences started to senesce, ca. 7 days after harvest as reflected from senescence score (Fig. 3.5C), the respiration rate increased slightly and remained at high level until the end of vase life. Respiration of BA-treated inflorescences was maintained at low level and it increased slightly toward the end of vase life.

In general, the respiration rate of some cut flowers decreases with time after harvest, then dramatically increases followed by a second decline as found in anthurium (Paull *et al.*, 1985), Easter lily leaf (Franco and Han, 1997), and carnation (Nichols, 1973b). This pattern of change in respiration is assumed to be analogous to the climacteric rise in respiration in many fruits where the peak in the respiration rate is considered to indicate the final senescence stage (Halevy and Mayak, 1979). For red ginger, the pattern of respiration change followed only the first part of the climacteric pattern, where the respiration decreased with time after harvest and then slightly increased toward the end of vase life (Fig. 3.5B). The rise in respiration at the end of vase life was concomitant with an increase in the senescence score (Fig. 3.5C), possibly indicating a disruption of the membrane structure and cytoplasmic organelles that normally occurs during senescence (Nichols, 1973b).

The results showed that BA delayed the rise in red ginger respiration rate, hence the inflorescence lasted longer than the untreated control. The effect of BA on delaying the rise in respiration rate has also reported for Easter lily leaf (Franco and Han, 1997). These findings indicated that red ginger inflorescences with a lower rate of respiration last longer than those with a higher rate.

Water balance of the cut stem: Water balance of cut stem can be determined as water uptake and water loss rate or transpiration (van Doorn *et al.*, 1995b). In general, water uptake and water loss fluctuate cyclically with an overall declining trend from harvest (Halevy and Mayak, 1981). This decline was also found for red ginger (Fig. 3.6A). Water uptake and water loss rate of both groups declined with time after harvest ($P = 0.0001$), the BA-treated inflorescence had values slightly higher than those of the control inflorescences. The slightly higher loss rates may result from the fact that BA can induce stomatal opening (van Staden *et al.*, 1988) in the inflorescence bracts, hence increasing the transpiration rate and water uptake of the cut stem. Increased water uptake of cut stem by application of cytokinins has been reported for several cut flowers, such as gerbera (van Meeteren and Gelder, 1980) and rose (Halevy, 1976).

For both untreated and BA-treated inflorescences, the rate of water uptake was almost the same as the water loss rate (Fig. 3.6A) resulted in a stable water balance (Fig. 3.6B) for the first 7 days after harvest. Inflorescence fresh weight changed only slightly during this period (data not shown). However, once water loss rate exceeded water uptake, about 7 days for control inflorescence and 12 days for BA-treated inflorescence, water balance turned negative. Control inflorescences developed senescence symptoms 4

days prior to BA-treated inflorescences as senescence scores started to increase (Fig. 3.6B). Once senescence started, control inflorescences developed symptoms faster than the BA-treated inflorescences and eventually ended their vase life 15 days before BA-treated inflorescences. The postharvest change in water balance was a mirror image of the senescence scores in which once the water balance turned negative, senescence scores started to increase (Fig. 3.6B).

Although there was a significant relationship ($r = -0.80$, $P = 0.0001$, $n = 390$, Table 3.8), between water balance and senescence score, this correlation cannot be concluded as a cause and effect relationship as they took place simultaneously. In cut rose, loss of petal turgidity and fresh weight is preceded by a decrease in the rate of water uptake, indicating that negative water balance is responsible for flower senescence (Durkin and Kuc, 1966). In gerbera, flower fresh weight loss decreased sharply 3 days before stem break occurs indicating that negative water balance is the cause for senescence (van Meeteren, 1978a). In red ginger, development of senescence symptoms (bracts turned brown and dry out) seems to be a major cause for fresh weight loss. Hence, negative water balance may result from senescence, rather than the cause as reported for other flowers.

Stem and vase microorganisms: A high number of microorganisms in a stem segment or in the holding solution decreases the rate of water uptake and shortens flower vase life (van Doorn *et al.*, 1989; van Doorn *et al.*, 1995b; Zagory and Reid, 1986a; Zagory and Reid, 1986b). In Europe, microorganism counts are required to be kept low at the grower level, but successively increase as the flower moves through the auction

system, wholesalers and retailers (Hoogerwerf and van Doorn, 1992). The major source of microorganisms in cut rose is from tap water (van Doorn and Witte, 1997). The number of microorganisms varies with plant samples, days after harvest, and handling systems. For instance, the number of microorganisms found under standard conditions for 5 different flowers are as follows: gerbera > rose > lily > Freesia > carnation, with the levels ranging from $\leq 10^4$ to $\geq 10^6$ cfu·g^{-1} (Hoogerwerf and van Doorn, 1992).

In red ginger, total counts one day after harvest were 10^5 cfu·g^{-1} FW and 3.6 x 10^3 cfu·ml^{-1} for stem segments and holding solution, respectively. The microbial count in stem segments increased sharply toward the end of vase life, with the maximum of 4 x 10^8 cfu·g^{-1} FW (P = 0.0001), whereas that in holding solution increased to the maximum (1.3 x 10^6 cfu·ml^{-1}) 9 days after harvest and then declined (Fig. 3.7A). The rate of water uptake by the same stems determined prior to stems being excised showed a significant negative relationship between total microorganisms count in the stem segments and water uptake (r = -0.60, P = 0.0002, n = 35, Fig. 3.7B).

Separate experiment was conducted to determine the number of total microorganisms in stem segment and vase solution compared between untreated and BA-treated inflorescences. In this study, total counts for microorganisms in stem segments increased to a maximum for both groups of samples, 2.2 x 10^8 and 4.6 x 10^8 cfu·g^{-1} FW for control and BA-treated inflorescences, respectively at day 12, after which it declined (Fig. 3.8A). The shrinkage of the stems and the water-soaking symptoms on some samples were possibly responsible for the decline in the total counts toward the end of the experiment.

Total counts for microorganisms in the vase solution was relatively low on the first day after harvest (0.2 x 10^5 cfu·ml^{-1} for both untreated and BA-treated inflorescences), but increased sharply on day 3 (2.9 and 3.9 x 10^5 cfu·ml^{-1} for control and BA treated inflorescences, respectively) (Fig. 3.8B). For untreated inflorescences, the total microorganisms in the vase solution increased to the maximum (3.7 x 10^5 cfu·ml^{-1}) on day 9, then declined. However, at the end of the experiment, the total microorganisms increased again. The increase in total microorganisms in the vase solution at the end of the experiment for the control inflorescence paralleled the increasing senescence scores (Fig. 3.8C) and the shrinkage of the observed stem, together with browning of inflorescence bracts. The accumulation of the microorganisms in the vase solution at the end of this experiment may results from migration of the microorganisms from the stem into the vase solution as the stem shrank and inflorescence dried. On the other hand, total microorganism count in the vase solution for BA-treated inflorescence increased to the maximum (5.9 x 10^5 cfu·ml^{-1}) on day 12, after which it declined (Fig. 3.8B), when the senescence score was low (Fig. 3.8C). Results showed vase life of BA-treated inflorescences was 1.5 folds lower than the control (Fig. 3.5C), although total counts of microorganisms in stem segments (Fig. 3.8A) and the vase solution (Fig. 3.8B) in BA-treated inflorescences were higher than in the control for most of the sampling dates. This result confirmed that the total count microorganisms, while negatively related to water uptake (Fig. 3.7B), had little direct effect on postharvest vase life of the BA-treated inflorescences. This finding suggested that a limitation or threshold may exist for microorganism count before it had an effect on red ginger vase life.

Although at least 25 different microorganisms are found in cut flowers (Zagory and Reid, 1986b), only few species have a significant effect on flower vase life and varies with plant species. In carnation, microorganisms that reduce flower vase life are a yeast, a fluorescent pseudomonad and a nonfluorescent pseudomonad (Zagory and Reid, 1986b), whereas microorganisms found most on dendrobium 'Pompadour' stem are *Bacillus sp.*, *Pseudomonad sp.*, and *Erwinia sp.* (Ketsa *et al.*, 1995). Unfortunately, in red ginger, only total count of the microorganisms was determined and the microorganisms not identified. The variation in specific microbial species on flower vase life as reported for other flowers could also explain why total count microorganisms had little direct effect on red ginger vase life. The effect of microorganisms on postharvest vase life of cut flower, therefore, may vary depending upon both plant and bacteria species.

Levels of total sugars and sucrose: The energy reserves of the cut stem are one indicator used to determine vase life of cut flowers and foliage (Halevy and Mayak, 1979). Leaf blackening of cut protea (*P. neriifolia* B. Br.) is the result of depletion of carbohydrate by the inflorescence due to the sugar demand for nectar production (Dai and Paull, 1995), and exogenous sugar is required for good Freesia opening after harvest (Sytsema-Kalkman *et al.*, 1995). In general, internal levels of sugars in mature flowers are higher than that in young or immature flowers (Sytsema-Kalkman *et al.*, 1995), and vary with plant tissues (Sacalis, 1973). Hence the stage of plant development could be one factor affecting vase life of cut flowers.

In red ginger, the energy reserves of a cut inflorescence were determined as the level of ethanol-soluble total sugar and sucrose. In general, total sugar was low when the inflorescences were immature and significantly increased with development (P =0.0001) (Fig. 3.9A). Total sugar in the bracts also increased with inflorescence development and at the full opening stage was 2.5 times higher than at the bud stage. Total sugar in the rachis increased with maturation and was significantly higher than that of the bracts for most development stages except for the full opening stage (P = 0.0001). Total sugar in the stem changed only slightly throughout development, while the level of sucrose was about 50% of total sugar at all stages of inflorescence development, except for the sucrose in the inflorescence stem (Fig. 3.9B). The level of sucrose in the stem increased with development (P = 0.0001). This finding suggested that inflorescences harvested at the immature stage would have a shorter vase life than those harvested at older stage due to a lower carbohydrate reserve (Fig. 3.2). The increase in sugar contents of the mature inflorescences may result from the increase in sink strength as the inflorescences start to open.

The postharvest changes in sugar were conducted using inflorescences harvested at the two-thirds open stage. In general, the level of total sugar decreased with time after harvest for both control and BA-treated inflorescences and in all studied tissues (Fig. 3.10A). Total sugar in the bract of BA-treated inflorescence was slightly higher than that of the control (P = 0.01). Although the levels of total sugar in rachis and stem significantly decreased with time after harvest (P = 0.0001), there was no significant difference in total sugars between the control and BA-treated inflorescences in all tissues.

The amount of sucrose in the bracts decreased with time after harvest ($P = 0.0001$, Fig. 3.10B). Although the rate of decrease seemed to be faster in the control than in BA-treated inflorescence, the level of sucrose in bract between control and BA-treated inflorescence was not significantly different. The rachis sucrose followed the same trends as the bracts with the sucrose declining with time ($P = 0.0001$), and no significant difference between control and BA-treated inflorescences. Sucrose in the BA-treated stem was significantly lower than that of the control ($P = 0.005$), and both significantly decreased after harvest ($P = 0.0001$).

Total sugar in the bract was relatively constant compared to that in rachis and stem for both untreated and BA-treated inflorescences. The level of sugar in the bracts of BA-treated inflorescence was significantly higher than that of the control, whereas the sucrose level of untreated stem was significantly higher than that of the BA-treated stem. This finding suggested that sucrose, as a major form of transported sugar in plant tissues (Kruger, 1990), moves from the red ginger stem into the bracts, as previously suggested for carnation and rose (Ho and Nichols, 1977; Kaltaler and Steponkus, 1974; Nichols, 1973b). The level of energy reserves of red ginger was a key factor for extending red ginger vase life treated with BA.

The translocation of internal sugar from the stem into the bracts, could be the same route as that of exogenous sucrose from the holding solutions. In the translocation of exogenous sugars, sucrose in the holding solution is first taken up by xylem (Chin and Sacalis, 1977a), and subsequently moves laterally between the xylem and phloem along the stem length, especially in the basal 10 cm of cut stem (Ho and Nichols, 1975).

Therefore, exogenous sucrose can translocate in both the xylem and phloem into the floral tissues. It should be noted that unlike an intact plant, the xylem transpiration stream and mass flow in phloem of cut flowers occur in the same direction, from basal to the distal end (Ho and Nichols, 1975).

Sucrose content of the BA-treated stem was significantly lower than that of the control (Fig. 3.10B) suggesting that sucrose translocated from the stem into the bracts occurred to a greater extent in BA-treated inflorescences than the untreated inflorescences. One mechanism that plant responds to exogenous cytokinin is to remobilize nutrients between treated and untreated tissues (van Staden *et al.*, 1988). BA-treated leaves remains green and continue to photosynthesize, whereas untreated leaves are yellowing because nutrients in untreated leaves are withdrawn to the BA-treated leaves (Leopold and Kawase, 1964).

Starch level: Postharvest changes in starch content have been less frequently determined. In red ginger, starch levels at one day after harvest were relatively higher in the rachis than in the bracts (Fig. 3.11). Starch level in bract was very low, and there was no significant difference between control and BA-treated bracts. In the rachis, starch level significantly declined after harvest ($P = 0.0001$) and BA-treated bracts had significantly higher level than the control ($P = 0.0001$). Starch in the stem was very high one day after harvest with BA-treated stem having a higher level than the control (Fig. 3.11). However, the level of starch in the stem declined sharply ($P = 0.0001$), and showed no significant difference between the control and BA-treated stem.

The postharvest decline in plant tissue starch is a common phenomenon reported for cut flowers such as rose (Marissen and Brijn, 1995), Freesia (Sytsema-Kalkman *et al.*, 1995; van Meeteren *et al.*, 1995), and *Sandersonia aurantiaca* (Eason *et al.*, 1997). The decline in stem starch from the first day may be due to the hydrolysis of starch to meet the high sugar demand of other tissues, as previous results showed a trend of sucrose translocation from the stem into the rachis and then to the inflorescence bracts (Fig. 3.10B). Cut roses held in a solution containing sucrose have higher petal starch than those held in water (Ho and Nichols, 1977). These findings suggested that exogenous sugar replaced the demand for starch hydrolysis, in order to maintain the sugar levels within the plant tissues. However, the hydrolysis of starch cannot account for the increase of sugar levels during Freesia opening (van Meeteren *et al.*, 1995), due possibly to the trace amount found in the inflorescence buds, suggesting other sources of sugar may be involved.

In red ginger, the hydrolysis of starch may occur firstly in the stem before other tissues because of its sharp decline after harvest. However, a significant relationship between starch content in stem sections and inflorescence vase life was not found in this study. The BA treatment increased inflorescence vase life from 12 days (control) to 21 days (Fig. 3.10), while average starch content in the stems was not significantly different between BA-treated and the untreated control.

3.4. Summary

Development of senescence symptoms during red ginger inflorescence senescence were classified as inflorescence wilting and the bract browning due to the discoloration of the bracts at distal end of the inflorescence. These senescence symptoms accounted for more than 90% of the senescence symptoms developed during red ginger senescence. The other symptom was darkening of the inflorescence bracts without wilting or bract browning at the apex.

Inflorescences harvested at more mature stages had longer vase life than those harvested at less mature stages. Stem diameter and a leaf attached to the stem had no effect on postharvest vase life of red ginger. Inflorescences with longer stems lasted longer than those with shorter stems, if the inflorescences were not subjected to packing. Packing inflorescences for two days with dry or moistened newspaper did not affect inflorescence vase life, if a plastic liner was used to prevent water loss from the stems, but vase life was shorter without the liners and it was always shorter than stem placed directly in water. After unpacking, holding solutions containing sucrose significantly increased inflorescence vase life over the DI water or solutions without sucrose, although rate of solution uptake was low. Ethylene inhibitors, AOA, STS, and MCP, did not extend red ginger vase life, suggesting that ethylene had little or no effect on the development of senescence symptoms in red ginger. Benzyladenine at 200 ppm, when applied as a postharvest spray to inflorescence bracts and attached leaf, increased red ginger vase life 1.2 folds over the untreated inflorescences.

Ethylene evolution increased slightly with time, but was very low compared to carbon-dioxide released by the respiration. Respiration rate decreased with time, but then increased during senescence. Inflorescences with a lower respiration rate (BA-treated) had a longer vase life than those with high a respiration rate (untreated). Although water balance showed a significant negative relationship with the senescence score ($r = -0.80$, $P = 0.0001$, $n = 390$) a cause and effect relationship could not be drawn, because the negative water balance occurred concomitantly with the senescence. The total count microorganisms for stem segments and the vase solution were 10^8 cfu·g^{-1} FW and 10^5 cfu·ml^{-1} for the stem and solution, respectively, but had no apparent effect on red ginger vase life.

Starch levels of the inflorescences significantly declined after harvest with the most significant decline occurring in the stem. However, there was no significant difference between BA-treated and control inflorescences in starch content. BA-treated rachis maintained significantly higher starch level compared to the control ($P = 0.0001$). However, there was no clear relationship between starch hydrolysis and postharvest vase life of red ginger. Total sugar was significantly higher in BA-treated bracts ($P = 0.01$), whereas sucrose level was significantly lower in BA-treated stem ($P = 0.005$) compared to untreated inflorescence, suggested translocation of sugar from the stem into the bracts was induced by BA treatment.

Two major factors were apparently involved in red ginger vase life. Long-lived inflorescences had a lower respiration rate and a higher level of total sugar in the inflorescence bracts compared to the shorter lived inflorescences. This finding suggested

that the maintenance of energy reserves in the cut stem after harvest was important for inflorescence longevity.

Table 3.1. Senescence symptoms and their occurrence during 4 month study without any application of postharvest treatment. The symptoms observed were DP, inflorescence wilting; CB, center bract browning symptom; and DK, bract darkening without drooping or browning.

Harvest Date	No. of Observation			% Symptom			Vase life (days)		
	DP	CB	DK	DP	CB	DK	DP	CB	DK
February 21, 1995	55	20	5	69	25	6	20b	25b	45a
March 14, 1995	38	32	0	54	46	0	19b	25a	-
April 11, 1995	13	60	7	16	75	9	21b	19b	41a
May 2, 1995	11	64	5	14	80	6	16c	21b	38a
Total/ Mean	117	176	17	38	57	5	19b	22b	41a

Means between the column within a row followed by the same letter were not significantly different ($P = 0.001$, n = 310).

Table 3.2. Vase life of young inflorescences (no bracts open) in 2% (w/v) sucrose holding solutions after 2 days of packing with moistened shredded newspaper.

Holding solutions	Vase life (day)		
	Packing		Mean
	Not-packed	Packed 2 day	
DI water	25a	27a	26
2% Solutions	16b	20b	18
Mean	21	23	

Means between and within the columns followed by the same letter were not significantly different ($P = 0.0005$, n = 8).

Table 3.3. Effect of 2-day packing, with moistened shredded newspaper, and stem length on vase life of red ginger. Inflorescences (10 inflorescences/treatment) were harvested each month for12 months. Data were presented mean for 12 months.

Packing treatment	Vase life (day)			
	Stem length			Mean
	30 cm	50 cm	70 cm	
Not packed	19.6c	24.8b	30.1a	24.8
Packed 2 days	18.3a	19.5a	20.5a	19.5
Mean	18.9	22.2	25.3	

Analysis of Variance	Pr > F
Packing treatment	0.0001
Stem length	0.0001
Packing x Stem length	0.01

Means within a row followed by the same letter were not significantly different.

Table 3.4. Effect of stem diameter, standard (stem diameter at the middle of stem ≥ 10.0mm) and small (stem diameter ≤ 10.0 mm), on vase life of red ginger after 2-day packing with moistened shredded newspaper. Experiments were performed twice. Data represented means from two experiments (mean ± sd, n = 30).

Criteria	Stem diameter	
	≥ = 10.0 mm	≤ = 10.0 mm
Flower length (cm)	21.6 ± 1.1	17.3 ±0.8
Stem diameter (mm)	12.2 ±1.1	9.4 ±0.7
Vase life (day)	15.6 ±3.3	16.5 ±2.3

Table 3.5. Effect of leaving a leaf attached to the stem on vase life of red ginger. Experiments were tested on both standard (stem diameter ≥ 10.0 mm) and small (stem diameter ≤ 10.0 mm) inflorescences.

Treatment	Vase life (day)		Mean
	Standard	Small	
Stem without leaf	26.5 ±14.2	22.3 ±8.5	24.4a
Stem with one leaf	26.7 ±6.9	26.0 ±8.6	26.4a
Mean	26.6a	24.2a	

Means between and within the columns followed by the same letter were not significantly different ($P = 0.05$, n = 20).

Table 3.6. Effect of packing materials and methods on vase life of red ginger. Experiment was carried out monthly for total of 12 months. Leaf vase life was evaluated separately on the same inflorescence based on inrolling of the leaf more than 50% of the leaf area. Data represented means for 12 months.

Treatment	Vase life (day)	
	Flower VL	Leaf VL
Not-pack	24.4a	17.5a
2-day dry pack without plastic liner	17.5b	11.8b
2-day dry pack with plastic liner	24.5a	15.4ab
2-day moistened pack without plastic liner	22.5ab	13.0b
2-day moistened pack with plastic liner	23.7a	13.8b
Analysis of Variance	Pr > F	Pr > F
Effect of plastic liner	0.032	0.067
Effect of moistened newspaper	0.251	0.878
Plastic x Moistened newspaper	0.123	0.225

Means within a column followed by the same letter were not significantly different (n = 40).

Table 3.7. Effect of benzyladenine (BA, 200 ppm spray) on vase life of the inflorescences, with and without the presence of one leaf attached to the stem. Leaf vase life was evaluated separately on the same inflorescence based on inrolling of the leaf more than 50% of the leaf area. The experiment was conducted twice and data represented means from the two experiments.

Plant Part	Vase life (day)		
	With leaf	Without leaf	Mean
Inflorescence vase life			
Control	26.3b	24.6b	25.6b
BA	32.1a	31.7a	31.9a
Leaf vase life			
Control			22.7b
BA			30.7a
Analysis of Variance		Pr > F	
Leaf		0.375	
BA		0.0001	
Leaf x BA		0.583	

Means within and between the column followed by the same letter were not significantly different (n = 20).

Table 3.8. Correlation coefficients and probability for correlation analysis between senescence score and water balance components for untreated inflorescences. Water uptake and water loss rates were determined as mg of water·g FW^{-1}·day^{-1}. Water balance was calculated by subtracting rate of water loss from water uptake rate. Senescence scores were evaluated based on senescence scale (Table 2.1). Ten inflorescences were used for each sampling date, and data were collected for total of 12 days (n =120). Raw data are shown in Appendix E.

Variable	Variable			
	Loss	Uptake	Balance	Score
Loss	1.00	0.88 (< 0.0001)	0.006 (0.95)	-0.09 (0.32)
Uptake		1.00	0.47 (< 0.0001)	-0.50 (< 0.0001)
Balance			1.00	-0.90 (< 0.0001)
Score				1.00

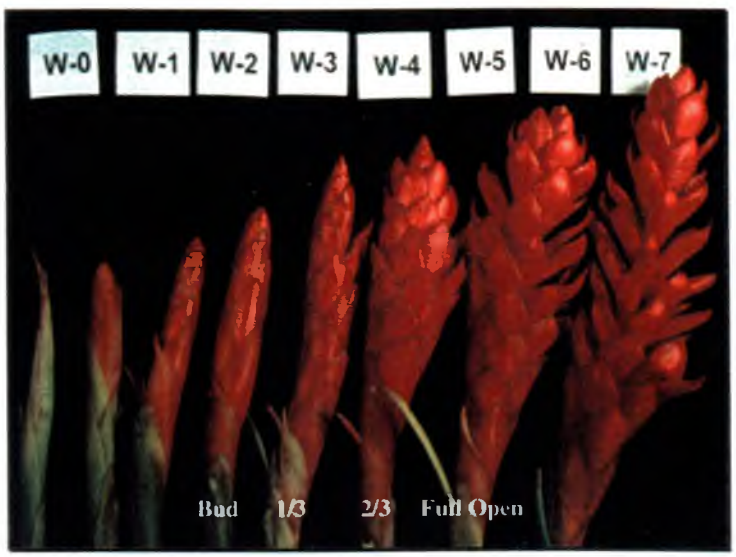


Figure 3.1. Developmental stages of red ginger inflorescence. Bud stage - refers to the inflorescence that had emerged 3 weeks (w-3) from the leaf sheath with no bracts open, 1/3 open stage - refers to when the 1/3 of total inflorescence bracts were open from the rachis (w-4), at the 2/3 open stage, 2/3 of total inflorescence bracts were open (w-5), and full when ¾ of the total inflorescence bracts had opened (w-6).

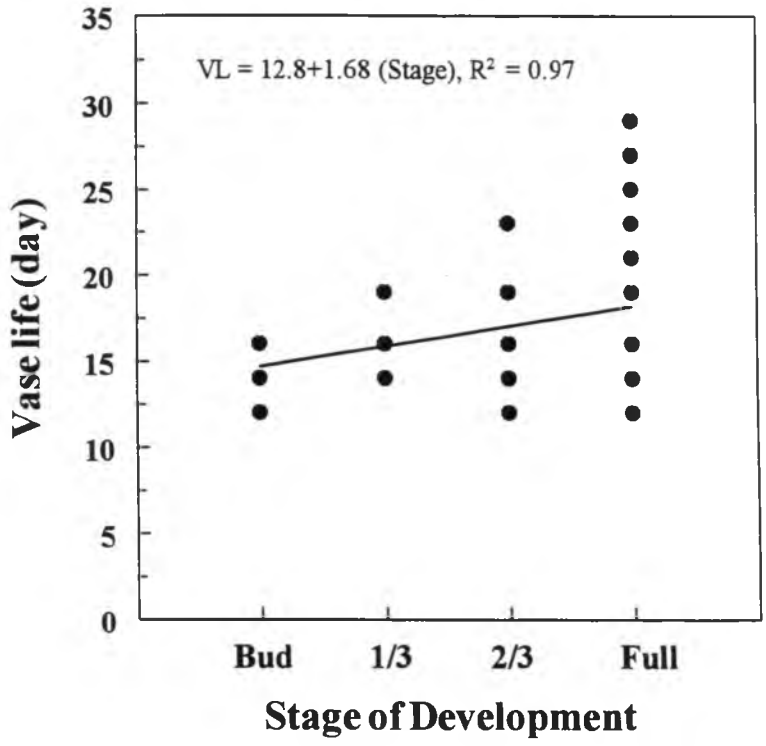


Figure 3.2. Postharvest vase life of red gingers harvested at different stages of development (n = 20). Bud stage refers to the inflorescence that has no open bract, 1/3 open refers to 1/3 inflorescence bract open, 2/3 open refers to 2/3 of inflorescence bract open, and full open refers to ¾ inflorescence bract open (Fig. 3.1). The older stage (full open, W-6) can last, on average, 5 more days than it cut at young stage (bud, W-3).

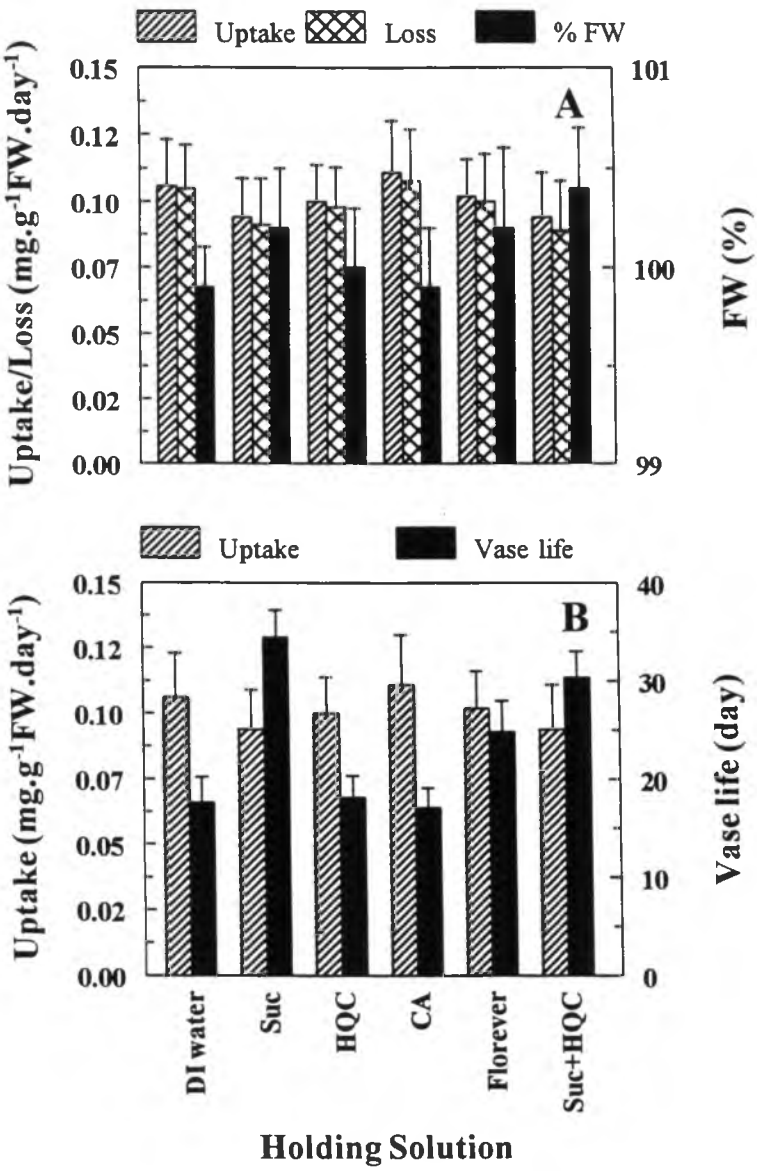


Figure 3.3. Rate of water uptake, water loss and percentage fresh weight change (A), water uptake and vase life of red ginger (B) held in different holding solutions. Solutions were deionized water, 2% (w/v) sucrose (Suc), 200 ppm Hydroxyquinoline citrate (HQC), 1 mM Citric acid (CA), 2% (w/v) Florever, a commercial preservative solution, and the combination between 2% (w/v) sucrose and 200 ppm HQC (Suc+HQC). Rate of water uptake, water loss rate and % FW were means from 7 days after harvest. For each treatment, 10 inflorescences without leaf sheath were used.

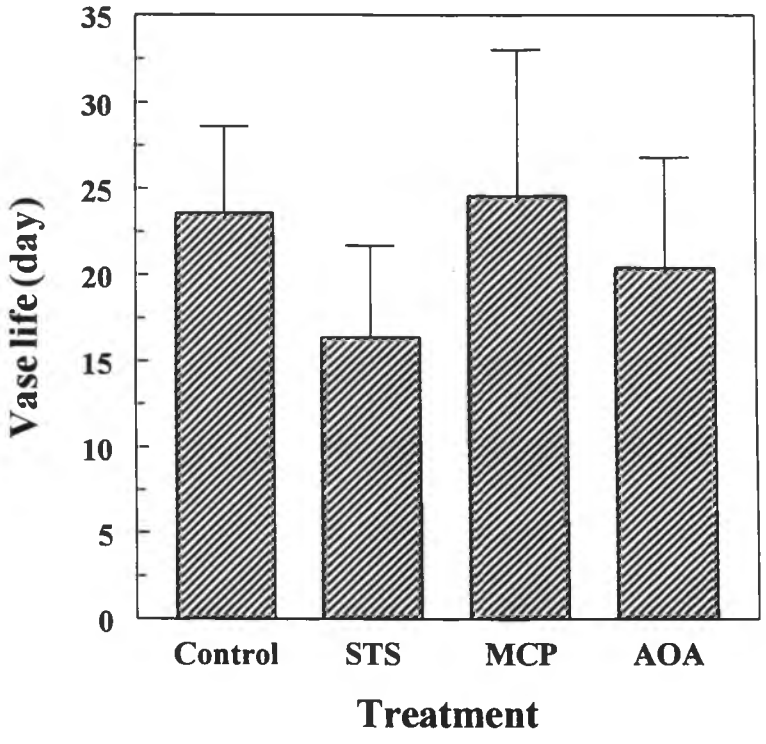


Figure 3.4. Effect of ethylene inhibitors on vase life of red ginger. Tested chemicals were 1mM: 4mM of silver nitrate: sodium thiosulfate (1:4, STS), 500 ppb 1-methylcyclopropene (MCP), and 25 μM amino-oxy acetic acid (AOA). Pulsing and gassing were applied for 6 h before being packed, whereas holding treatment was applied after unpacking. Twenty of 2/3 open stage inflorescences were used per treatment.

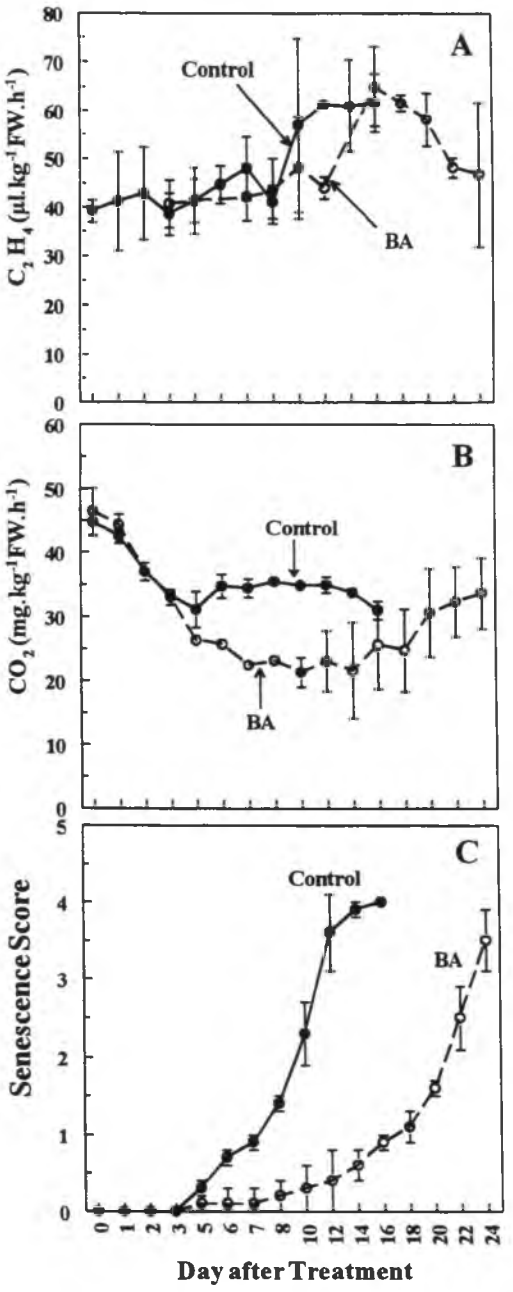


Figure 3.5. Changes in ethylene production (A), respiration rate (B), and senescence score (C) in untreated (•) and BA treated (o) inflorescences. Data were collected daily after experimental set up (Day 0) until end of each treatment vase life. Each data point represents mean from 3 respiratory chambers. Experiments were performed twice, and one experiment was selected to represent the results.

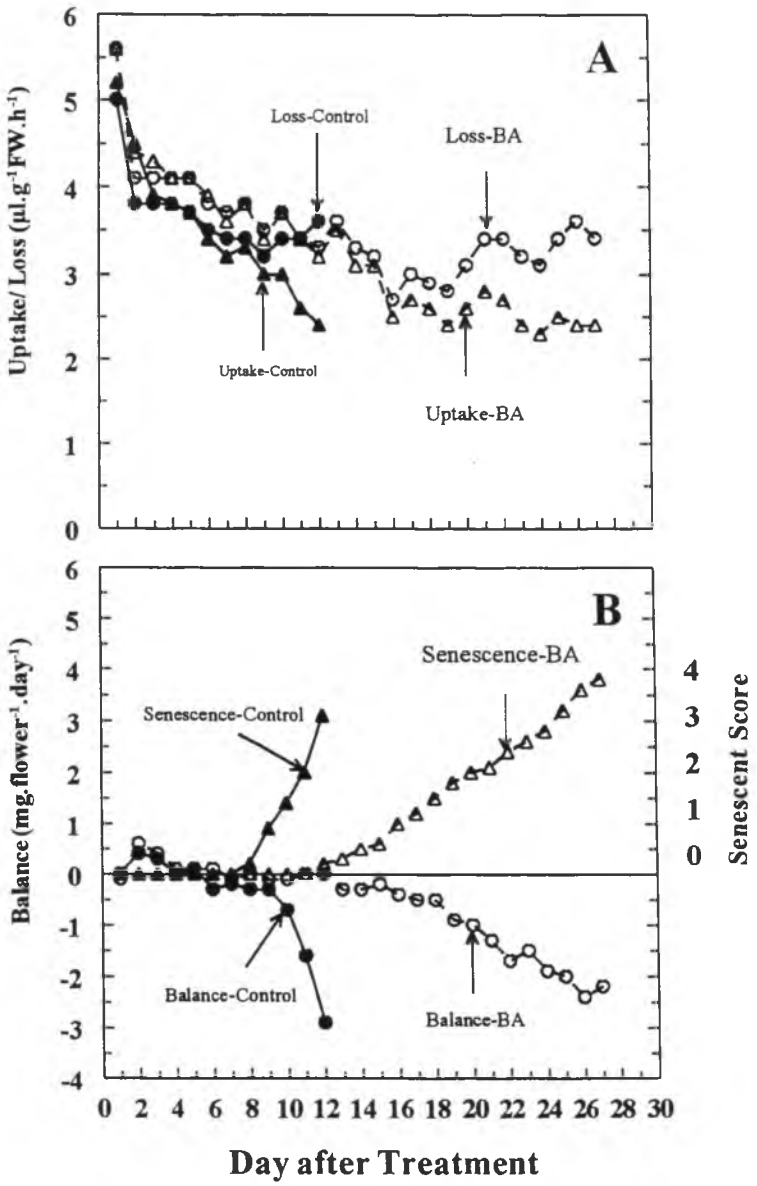


Figure 3.6. Changes in water uptake and water loss (A), water balance and senescence score (B) in untreated (•) and BA treated (o) inflorescences. Data were collected daily after one day of treatment until end of each treatment vase life. Each data point represents mean from 10 inflorescences. Experiments were performed twice, and one experiment was selected to represent the results.

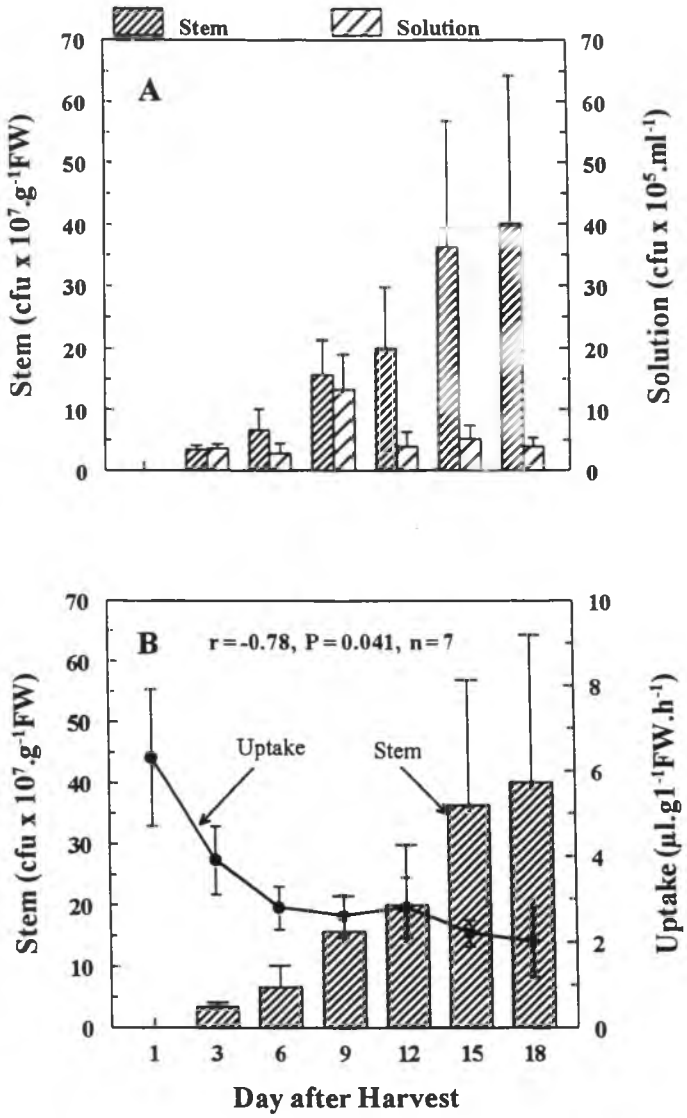


Figure 3.7. Total count microorganisms in stem segments and holding solution (A) and relationship between number of bacteria in the stem segment and the rate of water uptake (B). Samples were taken at 1, 3, 6, 9, 12, 15, and 18 days after harvest. For each sampling date, five stems were used per treatment. Experiments were performed twice, and one experiment was selected to represent the results. Unit was expressed as colony forming unit (cfu) per gram fresh weight (cfu·g^{-1}FW) or per solution volume (cfu·ml^{-1}).

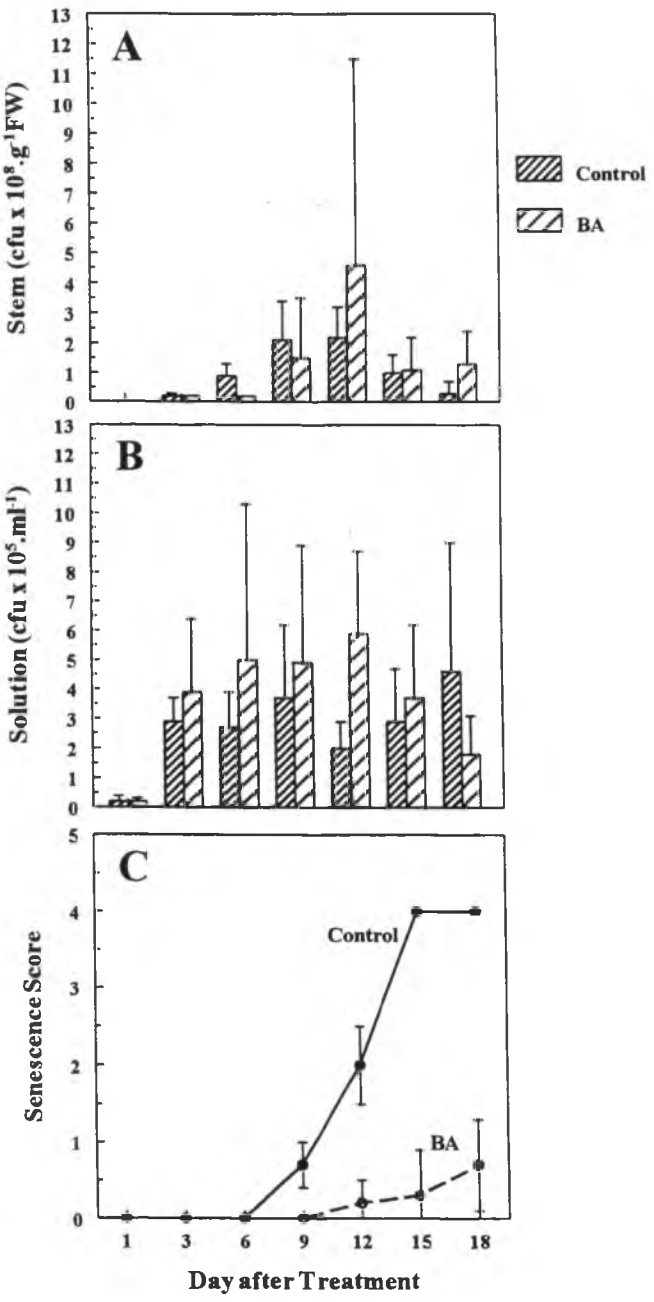


Figure 3.8. Total count microorganisms in stem segments (A), the holding solution (B), and senescence score (C) of untreated (•, or hatch) and BA treated (o, or light hatch) inflorescences. Samples were taken at 1, 3, 6, 9, 12, 15 and 18 days after treatment. For each sampling date, 5 stems were used per treatment. Experiments were performed twice, and one experiment was selected to represent the results.

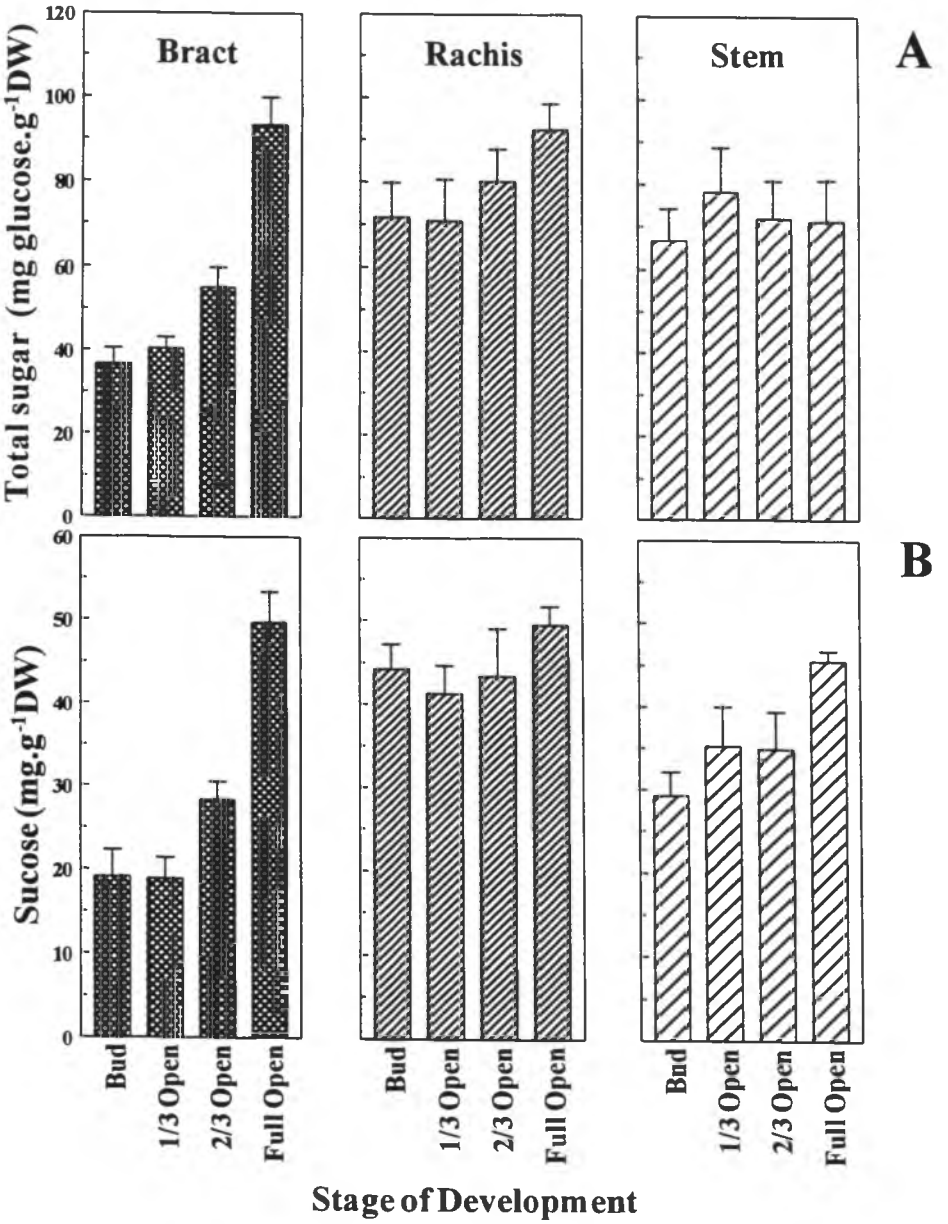


Figure 3.9. Total ethanol soluble sugar (A) and sucrose (B) in different plant parts at different developmental stages. Extraction of sugar was conducted 1 day after harvest. For each stage, samples were combined from 12 inflorescences and the extractions for sugars performed separately 3 times.

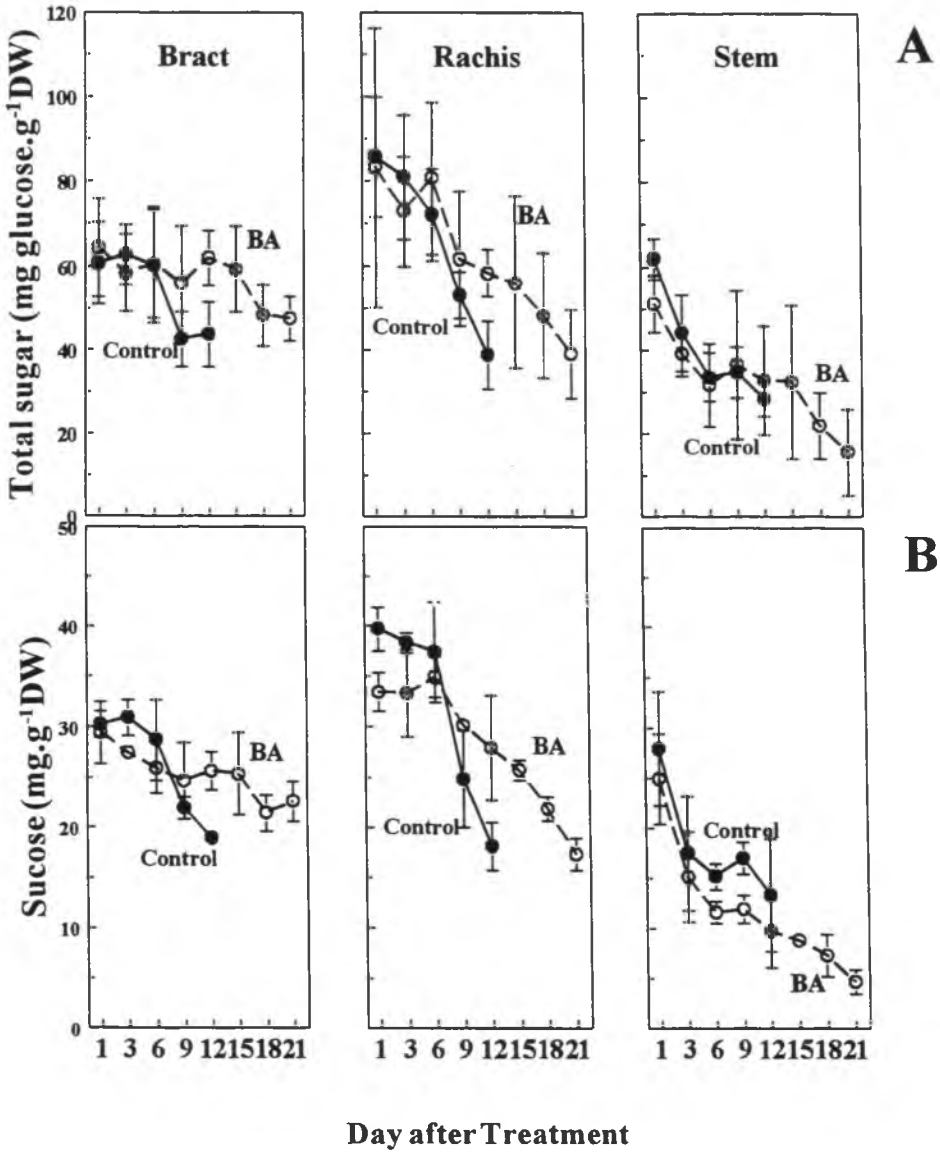


Figure 3.10. Changes in total ethanol soluble sugar (A) and sucrose (B) in different inflorescence parts of untreated (•) and BA treated (o) inflorescences. Inflorescences were harvested at 2/3 open stage. Sugar extractions were conducted at 1, 3, 6, 9, and 12 days after treatment for untreated inflorescences, and up to 15, 18, and 21 days for BA-treated inflorescences. For each sampling date, samples were combined from 5 inflorescences, and sugar extractions were performed separately 3 times.

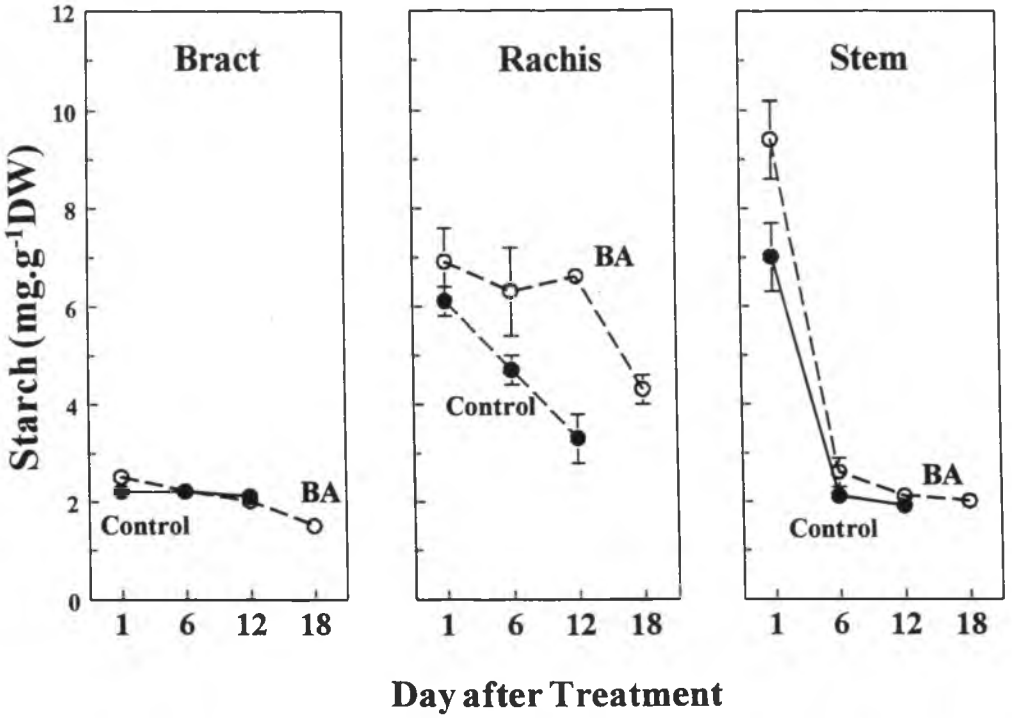


Figure 3.11. Changes in starch content in different inflorescence parts of untreated (•) and BA treated (o) inflorescences. Inflorescences were harvested at 2/3 open stage. Starch extractions were conducted at 1, 6, and 12 days after treatment for untreated inflorescences, and up to 18 days for BA-treated inflorescences. For each sampling date, samples were combined from 5 inflorescences, and starch extractions were performed separately 3 times.

CHAPTER 4

HOT WATER AND RED GINGER VASE LIFE

4.1. Introduction

Hawaiian tropical cut flowers, including red ginger, must be subjected to insect disinfestation before shipment to the mainland US. Treatments such as hand removal, irradiation, fumigation, insecticidal dips, temperature treatments and the use of biological control agents have been tested (Hansen and Hara, 1994). Red ginger inflorescences are currently hand-washed and dipped in insecticidal solutions. However, these procedures are labor intensive. A hot water treatment has been developed to control insects in tropical cut flowers and foliage (Hara *et al.*, 1997; Hara *et al.*, 1996; Tenbrink *et al.*, 1992). Temperatures and exposure times required for quarantine treatments vary depending on insect and plant species. The hot water treatment (49°C for 12 min) developed for insect disinfestation has been reported to extend red ginger vase life. Vase life of red ginger was thought to correlate with preharvest factors, such as rainfall and field temperatures before harvest (Hara *et al.*, 1997; Hara *et al.*, 1996). However, the responses of red ginger to different ranges of temperatures and exposure time have not been studied. For example, the threshold limitation for hot water treatment causing inflorescence damage and whether preconditioning treatments can reduce inflorescence damage are unknown. This study was undertaken to determine the threshold for heat damage, preconditioning temperatures before high temperature exposure, and the effect of preharvest factors on inflorescence vase life.

4.2. Materials and methods

4.2.1. Plant materials

Red ginger inflorescences cv. 'Red ginger' were harvested from plants grown at Poamoho Experiment Station on the Island of Oahu, except for the experiment conducted to test effect of a selective treatment in 1997 when a red ginger cv. 'Raspberry' from Waimanalo Experiment Station, on the same island, was used. All experiments were conducted between May 1995 and December 1997. General handling procedures were described in Chapter 2. After culled for uniformity, stems (with one leaf) were cut to the same length (55 cm). In general, the preparation of the plant materials including washing of the unheated inflorescences was done in the afternoon, and the hot water treatments were applied on the following day (one day after harvest). The dryness of the washed materials and the hot water treated inflorescences was carried out at room temperature ($23 \pm 2°C$) by allowing the inflorescences to stand in the buckets containing tap water for 6 to 12 h. After dryness, all inflorescences were packed for two days, using moistened, shredded newspaper with plastic liner, to simulate shipping. After unpacking, 5 cm of the cut end was removed under water and the inflorescences were placed in the buckets containing tap water for evaluation of heat damage vase life. All inflorescences were evaluated individually.

Inflorescence bract damage due to hot water treatment was evaluated on a scale of 0 to 4 (Fig. 4.1, Table 4.1), 5 to 7 days after unpacking. Inflorescences with a damage score of 3 or 4 were discarded as having ended their vase life due to the loss of

appearance. Inflorescence condition was evaluated every other day based on senescence symptoms as described previously (Table 2.1)

4.2.2. Hot water treatments

Inflorescences were bunched into 10 per treatment, and each inflorescence represented a replication. Twenty inflorescences were submerged in a circulating hot water tank (277.4 liters) at a time. Stem and water temperature was monitored with a thermocouple data logger ECD-model 5100 (Electronic Controls Design, Milwaukee, OR). The hot water treatments tested were 45, 47, 49, 50, 51, 52, and 55°C. Water temperature was maintained to ± 0.2°C by the addition of hot or cold water to the tank. Exposure times ranged from 2.5 to 30 min. Immediately following each treatment, inflorescences were hydrocooled in tap water at 23 ± 2°C for 15 min. After hydrocooling, the inflorescences were allowed to dry with the stem standing in a bucket of tap water for 6 to 12 h. Control inflorescences were submerged in tap water containing 7.5 $g \cdot L^{-1}$ Liqui-Nox detergent (Alconox Inc., N. Y.) for 15 min to eliminate insect infestations. After washing, inflorescences were rinsed with tap water for 15 min. Preliminary results (data not presented) showed that the detergent did not affect inflorescence vase life.

The threshold for hot water application was determined by regression analysis of the exposure times on the hot water temperature treatments that caused slight damage to inflorescence bracts.

4.2.3. Preconditioning treatments

Submerging the inflorescences in warm water in an effort to induce thermotolerance was the preconditioning treatment. Three preconditioning temperatures (35, 37.5, 40°C) and three exposure times (15, 20, 30 min) were tested. Following each preconditioning treatment, inflorescences were held for one hour at room temperature (ca. 22°C) then exposed to hot water (50°C for 15 to 25 min). In a second experiment, the length of the period between the preconditioning (40°C for 30 min) and the hot water treatment (50°C for 15, 20, or 25 min) was varied (1, 3, 6, 9 h). Following the hot water, all procedures for packing and unpacking were as described above.

4.2.4. Preharvest factors and heat sensitivity

In the first year of monitoring (January to December 1996), 'Red ginger' cultivars were harvested monthly from Poamoho Experiment Station, whereas in the second year of monitoring (January to December 1997), 'Raspberry' cultivars were harvested monthly from Waimanalo Experiment Station. Data were analyzed and compared between the two cultivars.

To determine the effect of preharvest environmental factors on inflorescence heat sensitivity, inflorescences were preconditioned at 40°C for 15 min, left to dry while standing in water for one hour, and then treated with hot water at 50°C for 12 min. Correlation analysis was run for total accumulated of rainfall (cm), average maximum and minimum temperatures, damage scores, and vase life in order to determine if there was an effect of abiotic factors on inflorescence vase life and heat sensitivity.

4.3. Results and discussion

4.3.1. Observation of heat damage symptoms

Exposure of leafy vegetables and ornamentals to elevated temperatures produces heat stress that leads to injury, such as wilting and browning (Paull and McDonald, 1994) or cell death (Levitt, 1980). The limit of heat tolerance is dependent on both temperature and exposure time. In red ginger, heat damage symptoms were scalding and necrosis of the inflorescence bracts. In general, necrosis progressed inward as the degree of damage increased until the bract was completely brown. Bracts were regarded as having severe damage when ≥ 30% of the total bract area showed damage. Leaf-scalding and inward-rolling occurred only when inflorescences were subjected to treatments that caused severe damage. Observation of unpacked materials showed that bracts suffering severe or extreme damage (scale 3 or 4, Table 4.1), showed damage symptoms within 24 h; moderate symptom after 2 to 4 days; and slight damage within 5 to 7 days after the hot water treatment.

4.3.2. Hot water treatments

Preliminary experiments had shown that ca. 5 min was required to raise the stem temperature to the water temperature and that 12 to 15 min of hydrocooling was needed to bring stem temperature back to ambient temperature (23 ± 2°C) (Fig. 4.2). The most effective temperature for insect disinfestation is between 47 to 49°C (Hara *et al.*, 1996; Tenbrink *et al.*, 1992). However, in this study, hot water at 49, 50, or 51°C (Fig. 4.3) showed a higher potential to increase inflorescence vase life than 45, 47, 52, and 55° C,

(Fig. 4.4). This result confirmed the previous results of Hara *et al.* (1996; 1997) that 49°C for 12 min extended vase life of red ginger. The peak of the responses occurred at exposure times of approximately 10 to 20 min, 5 to 15 min, and 5 to 10 min for hot water temperatures of 49°C, 50°C, and 51°C, respectively.

Short exposure times at high temperatures (7.5 min at 52°C and 2.5 min at 55°C) caused severe damage to the inflorescence bracts. The long exposure times at low temperatures (15 and 30 min at 45°C and 10, 20, and 30 min at 47°C) cause no damage to the inflorescence bracts, but vase life of the inflorescences was not extended (Fig. 4.4). This data suggested that short exposure to near lethal temperatures showed a higher potential to increase vase life than a longer exposure to lower temperatures.

4.3.3. Threshold for hot water treatments

Because of inflorescence availability, the threshold for inflorescence bract damage was investigated twice for the cultivar 'Red ginger' in two different seasons and years: summer 1995 (May to August) and winter 1997 (February to March). The threshold line was plotted through the values corresponding to slight to moderate damage on inflorescence bracts. Points below and above the threshold line represented 'no damage' and 'severe damage' on the inflorescence bracts, respectively. Results showed that summer (Fig. 4.5) and winter (Fig. 4.6) thresholds, as represented the relationship between temperatures (T, °C) and logarithm of exposure times (Z, min), were straight lines and had similar slopes. There was a shift of winter threshold toward the less severe treatment (lower temperature or shorter exposure time) (Fig. 4.7) indicating that

inflorescences in winter were less thermotolerance than inflorescences harvested in summer.

Heat damage to plants is strongly time-dependent. The damage relationships between temperature and exposure time for red ginger inflorescence were similar to that reported for leaves of *Hepatica nobilis* as the logarithm of exposure time for injury versus stress temperature in leaves was a straight line (Alexandrov, 1964). A logarithmic relationship occurs between temperature and exposure time for heat-killing of several plants, such as *Tradescantia discolor*, *Beta vulgaris*, *Brassica oleracea*, and *Pisum sativum*. The relationship between heat-killing temperature (T) and exposure time (Z) in these plants is represented by straight-line equation T = a - b Log Z, where a and b are constants (Levitt, 1980).

The shifting of winter threshold toward the less severe treatment indicated that heat tolerance in red ginger exhibits a seasonal cycle that is high during summer and low during winter. The increase in heat tolerance during summer may result from thermal hardening of plant tissues due to high environmental temperatures (Levitt, 1980).

4.3.4. Preconditioning treatments and intervening period

Hot water (49 to 51°C) extended red ginger vase life with only slight damage to the bracts (Fig 4.4; 4.5). Although this damage did not accelerate with inflorescence senescence, it reduced the aesthetic values of the inflorescence bracts. To reduce bract damage, preconditioning treatments were investigated by subjecting the inflorescences to lower temperatures prior to the hot water treatments. Preconditioning commodities at

temperatures lower than actual heat treatment temperature to induce thermotolerance has been reported for papaya (Paull and Chen, 1990), cucumber (Chan and Linse, 1989), and red ginger (Hara *et al.*, 1997; Hara *et al.*, 1996). Hot air was used to precondition cucumber (32.5°C for 24 h) and red ginger (39°C for 2 h) and hot water for papaya (42°C for 30 min). Although water is more effective than air as a heat transfer medium and quickly establishes a uniform temperature profile (Armstrong, 1994), preconditioning with hot water has not been previously reported for the red ginger.

Preconditioning at 40°C showed a higher potential to reduce bract damage than preconditioning at 35°C or 37.5°C when hot water was tested at 50°C for 25 min (Fig. 4.8A). Three exposure times (15, 20, or 30 min) at 40°C did not show significant differences in reducing bract damage, especially when compared to hot water tested at 50°C for 15 min (Fig. 4.8B). However, when hot water was tested for 25 min, a positive relationship was observed as the longer the exposure time at 40°C, the higher potential to prevent bract damage (Fig. 4.8B).

The thermotolerance characteristics of plant tissues are reported to correlate with low molecular weight heat shock proteins (HSPs) (Chou *et al.*, 1989; Yen *et al.*, 1994) that are normally synthesized when the plant tissues are subjected to elevated temperature not in the lethal ranges. Thermotolerance of the pear cell culture was induced by exposure to heat shock at 38 °C for 20 min, and it was maintained up to 6 h after which it declined (Wu and Wallner, 1984).

Results from intervening period study between preconditioning (40°C for 15 min) and the hot water treatment (50°C for 12 min) showed that red ginger lost its ability to

tolerate hot water treatment if the intervening period was longer than 6 h (Fig. 4.9). This result suggests that the loss of thermotolerance ability in red ginger after the preconditioning may be associated with heat shock proteins induced thermotolerance as indicated by the study in pear suspension culture (Wu and Wallner, 1984).

4.3.5. Preharvest factors and heat sensitivity

Large variation was found with vase life of the inflorescences harvested at different times of the year, within a cultivar (Fig. 4.10A, B). The response of red ginger to hot water varied greatly with cultivars. In 'Red ginger', hot water showed greater effect to extend vase life in the dry season (March to August) than the wet season (September to February) (Fig. 4.10A). This seasonal variation could be due to field-induced thermotolerance (Paull and McDonald, 1994). By contrast, the cultivar 'Raspberry', showed no extension of inflorescence vase life by the same treatment (Fig. 4.10B). This result suggested that there were difference between these two cultivars in thermotolerance, as reported for fruit cultivars in thermotolerance (Paull and McDonald, 1994).

The study of correlations between preharvest factors and red ginger vase life provides just guidance to develop an understanding of the possible effect of preharvest factors on postharvest vase life. Under field conditions, these factors cannot be controlled, but by looking at the collected data, at a certain period of time before harvest, the correlation may help to estimate the possible effects of hot water treatment on bract damage or inflorescence vase life.

Damage score from hot water treatment showed strong negative correlation with average of minimum (night) temperature 7, 14, and 21 days before harvest ($P = 0.02$), with maximum (day) temperature 14 days before harvest ($P = 0.03$), and with average temperature 14 days before harvest ($P = 0.03$) (Table 4.2). These results suggested that low temperatures during the inflorescence development (7 to 21 days before harvest) could induce a high damage score. The temperatures in the correlative period ranged from 14.5 to 22.2°C for night temperature, 22.0 30.5°C for day temperature, and 18.8 to 26.2°C for average temperature. On the other hand, a strong positive correlation was found for damage score and accumulation of rainfall 7 ($P = 0.004$) and 14 days ($P = 0.003$) before harvest (Table 4.2). These results suggested that during 2 weeks before harvest, if the accumulation of rainfall was high, the chance to have bract damage when applying hot water was also high. The accumulated rainfall in correlative period ranged from 0.06 to 18.9 inches and 0.06 to 26.2 inches for 7 and 14 days, respectively. The high damage score occurred during period of high rainfall of 7 to 14 days before harvest (Appendix F) might result from fully hydrated cells being less tolerant to severe heat treatment compared to the slightly less hydrated state (Kappen, 1981; Levitt, 1980).

No significant correlation existed between these preharvest factors and inflorescence vase life, except for the accumulation of rainfall 30 days before harvest. Accumulation of rainfall 30 days showed a strong negative correlation with inflorescence vase life ($P = 0.04$, Table 4.2), when rainfall ranged from 0.63 to 27.7 inches, and vase life varied from 10.3 to 34.3 days. This data suggested that high amount of rainfall

during the inflorescence development (30 days before harvest), could shorten inflorescence vase life after the hot water treatment.

The effect of hot water treatment on red ginger vase life reported in this study was similar to that reported by Hare *et al.* (1996; 1997). In their experiments, a single hot water treatment and exposure time was investigated (49°C for 12 min). However, our data showed that longer exposure time (15 min) at higher temperature (50°C) also extended red ginger vase life. This difference in temperature and exposure time may result from different preharvest environmental conditions at the different production areas and the procedure to precondition inflorescences. While preconditioning used warm water at 40°C for 15 min in this study, Hara *et al.* (1996; 1997) used hot air at 32°C for 2 h to induce red ginger thermotolerance. The inflorescences used by Hara *et al.* (1996; 1997) were grown in an area, Waiakea, Island of Hawaii, that received 3 to 4 fold more rainfall than most areas on Oahu and may, therefore, be more hydrated than inflorescences from our site. Hence, inflorescences may be subjected to high damage during heat treatment (Kappen, 1981).

4.4. Summary

Different ranges of temperatures and exposure times were studied to determine suitable hot water treatments to extend red ginger vase life. Hot water at 50°C for 12 to 15 min showed a high potential to prolong vase life by up to 15 days over control inflorescences. Relationship between temperature (°C) and logarithm of exposure time (min) for treatment causing slight bract damage (≤ 5 % of total bracts area) represented

by a straight-line equation: Temp = 55.23 - 4.5 log exposure time, R^2 = 0.97 (P = 0.0001). The threshold line shifted toward a less severe treatment (lower temperature or shorter exposure time) when treatments were applied during cool period.

The analysis of preharvest environmental correlation yield a strong negative correlation between damage score and field temperatures up to 21 days before harvest, and positive correlation between damage score and accumulated rainfall 7 days and 14 days before harvest. The negative correlation between inflorescence vase life and preharvest factors was found only for the accumulated rainfall 30 days before harvest. The possible interpretation of these analysis include: inflorescences harvested when night, day, or average temperature were low during the 14 to 21 days before harvest could result in high damage score. The high accumulation of rainfall during the 14 days before harvest could induce high damage score, and high rainfall during inflorescence development (up to 27.7 inches within 30 days before harvest) could shorten inflorescence vase life after the hot water treatment.

Preconditioning inflorescences at 40°C for 15 min before hot water (50°C, 12 to 15 min) significantly reduced damage from the hot water treatment. Preconditioned inflorescences lost their tolerance to the hot water treatment if the intervening period between preconditioning and the hot water was longer than 6 h. The suggested treatment to extend vase life is preconditioning at 40°C for 15 min, standing in the bucket of water at room temperature (22 ± 2°C) for one h, and then a hot water treatment at 50°C for 12 to 15 min. After hot water treatment, inflorescences should be cooled down in running

water for another 15 min. The ability of hot water treatment to extend red ginger vase life was found to be cultivar dependent.

Table 4.1. Criteria used to evaluate red ginger bract damage from hot water treatment. Inflorescences were evaluated as end of vase life when the damage score was ≥ 3.

Scale	Description	Criteria
0	No damage	No scalding or necrosis of inflorescence bracts
1	Slight damage	Scalding or necrosis only at the edge of the bracts, ≤ 5% of total bract area damaged
2	Moderate damage	5 – 10% of total bract area damaged
3	Severe damage	10 – 30% of total bract area damaged
4	Extreme damage	≥ 30% of total bract area damaged

Table 4.2. Correlation analysis between inflorescence vase life, difference in vase life between unheated and hot water (50°C, 12 min) heated inflorescences, damage score, total rainfall, and average field temperatures at different length of times before harvest. Data were collected monthly from January to December 1996 and pooled for analysis (n =12). Raw data (means from 10 inflorescences) are shown in Appendix F.

Days before harvest	Correlation Coefficient, r (probability, P)		
	Damage score	Vase life	Difference in vase life
Total Rainfall			
7	0.76 (0.004)	-0.16 (0.69)	0.067 (0.84)
14	0.77 (0.003)	-0.25 (0.52)	0.009 (0.98)
21	0.60 (0.04)	-0.63 (0.07)	-0.19 (0.55)
30	0.49 (0.11)	-0.68 (0.04)	-0.21 (0.51)
Minimum Temperature			
7	-0.75 (0.01)	-0.37 (0.29)	-0.38 (0.27)
14	-0.72 (0.02)	-0.35 (0.32)	-0.42 (0.23)
21	-0.70 (0.02)	-0.54 (0.10)	-0.56 (0.09)
30	-0.51 (0.13)	-0.68 (0.03)	-0.71 (0.02)
Average Temperature			
7	-0.59 (0.07)	-0.29 (0.42)	-0.39 (0.26)
14	-0.67 (0.03)	-0.30 (0.41)	-0.43 (0.22)
21	-0.60 (0.07)	-0.41 (0.24)	-0.54 (0.10)
30	-0.42 (0.22)	-0.56 (0.09)	-0.68 (0.03)
Maximum Temperature			
7	-0.58 (0.08)	-0.21 (0.56)	-0.35 (0.32)
14	-0.67 (0.03)	-0.22 (0.54)	-0.38 (0.28)
21	-0.51 (0.13)	-0.25 (0.49)	-0.44 (0.20)
30	-0.37 (0.29)	-0.43 (0.22)	-0.61 (0.64)

Figure 4.1. Rating scale for bract damage from hot water treatment (Table 4.1).

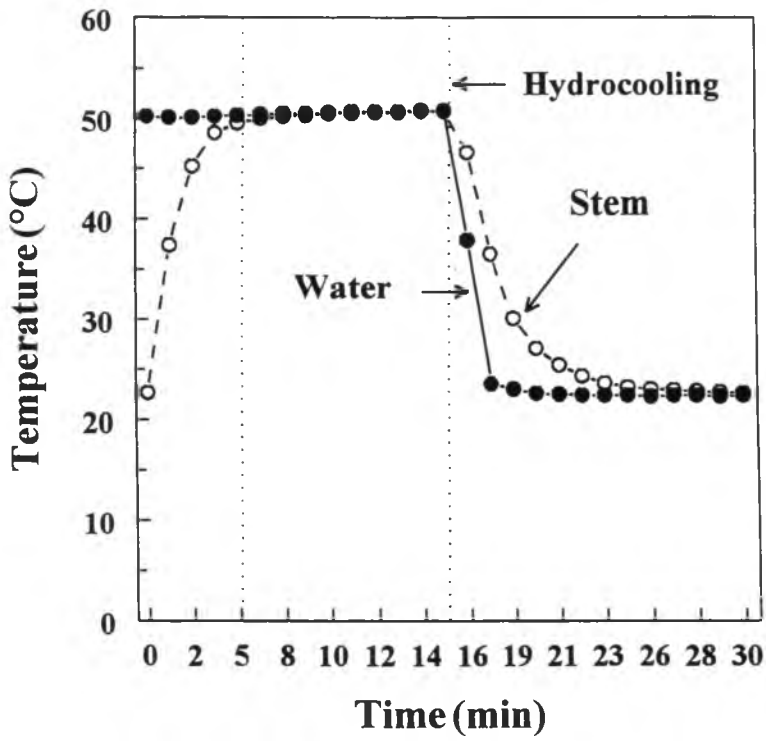


Figure 4.2. Water (•) and stem (o) temperatures after being subjected to hot water at 50°C for 15 min, before hydrocooling for another 15 min. Twenty of 2/3 open stage inflorescences with a leaf attached to the stem were submerged in to the hot water tank. Each data point represents mean from 10 temperature probes. Vertical dot lines indicate times when stem temperatures reached 50°C, and when hydrocooling was applied.

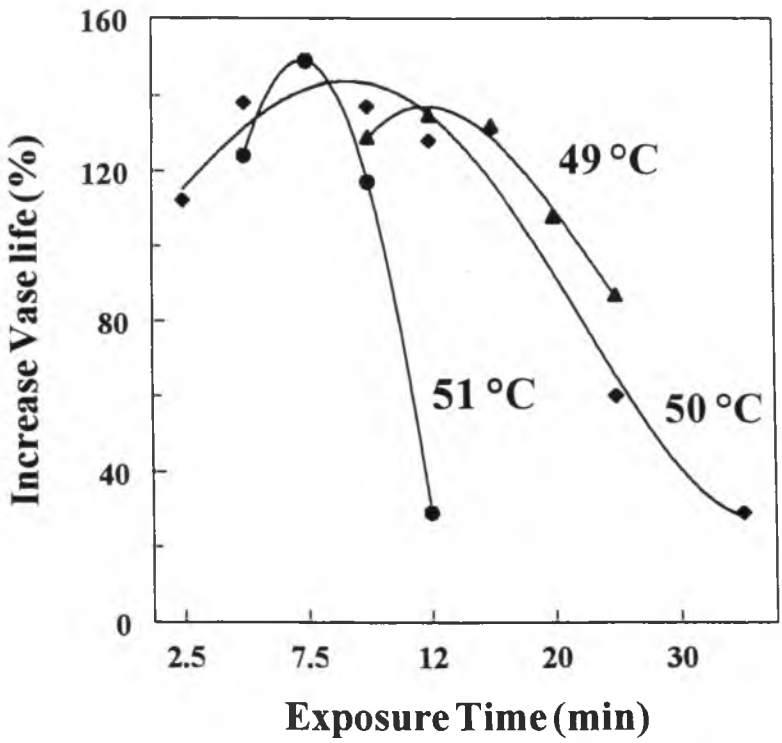


Figure 4.3. Effects of hot water treatments at 49°C, 50°C, and 51°C on red ginger vase life. Ten of 2/3 open stage inflorescences were used per treatment. Hot water treatments were applied 1 day after harvest. Experiments were performed at least twice and conducted separately for each temperature due to inflorescence availability. Control treatments were included in all experiments to allow comparison between experiments. Data for each temperature represent the percentage for the control in that experimental run.

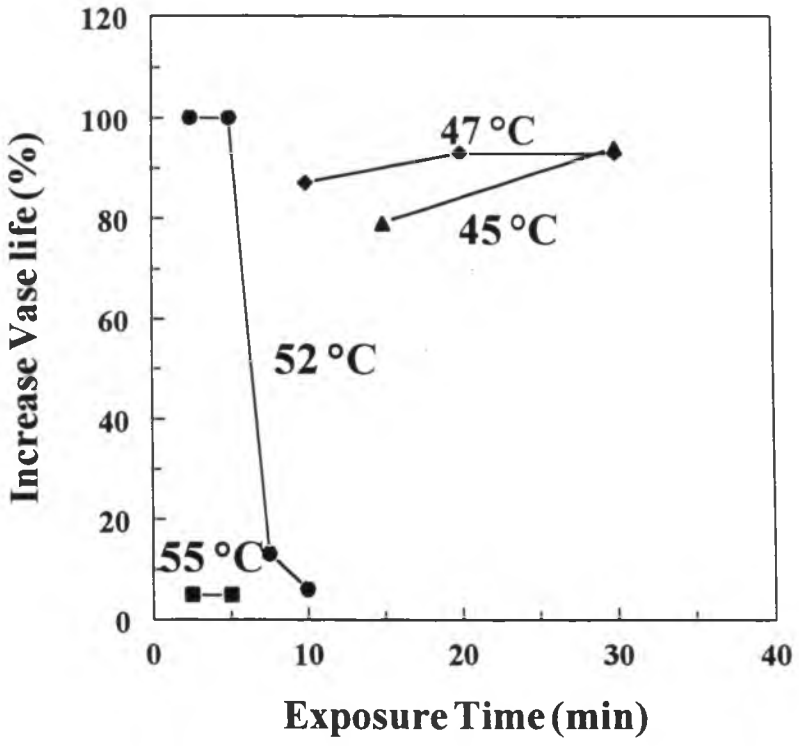


Figure 4.4. Effects of hot water treatments at 45°C, 47°C, 52°C, and 55°C on red ginger vase life. Ten of 2/3 open stage inflorescences were used per treatment. Hot water treatments were applied 1 day after harvest. Experiments were performed at least twice and conducted separately for each temperature due to inflorescence availability. Control treatments were included in all experiments to allow comparison between experiments. Data for each temperature represent the percentage for the control in that experimental run.

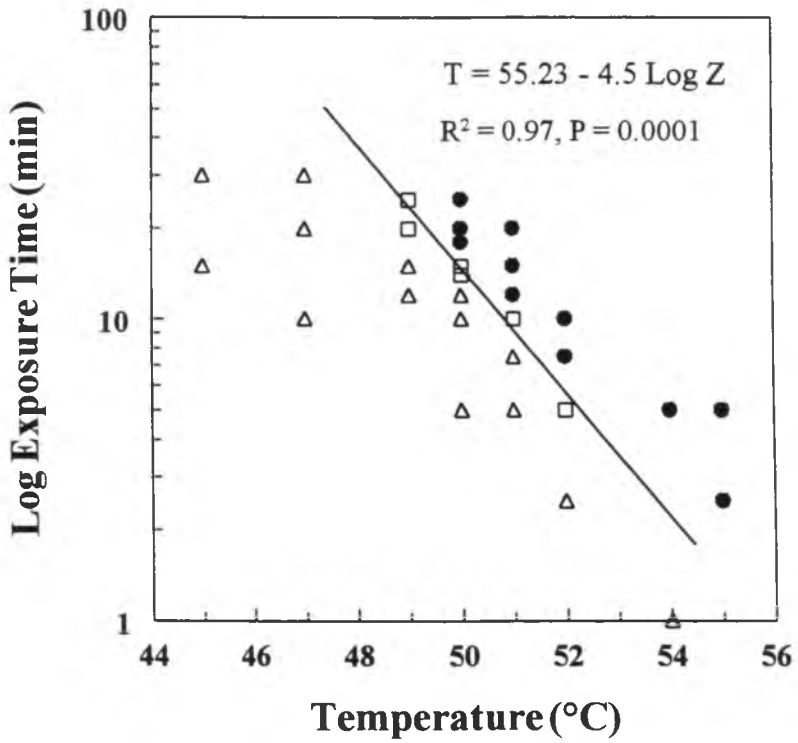


Figure 4.5. Relationship between temperature (T) and log of exposure time (Z) for the development of severe (•), slight (□) and no damage (Δ) on red ginger inflorescences conducted in summer 1995. Equation represents the regression for slight damage symptoms only. Each data point represents mean from 8 to 10 inflorescences. Experiments were conducted separately due to flower availability.

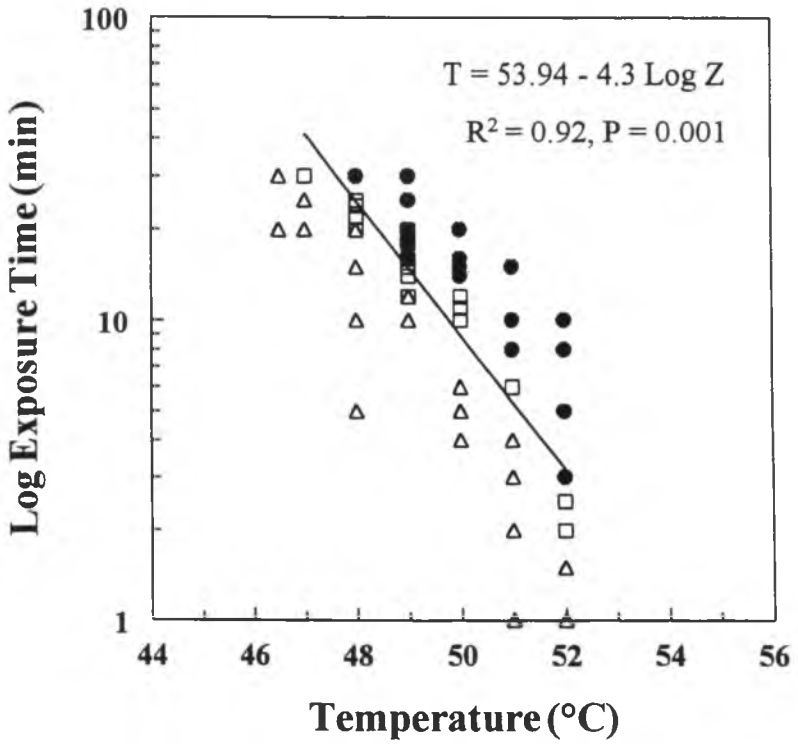


Figure 4.6. Relationship between temperature (T) and log of exposure time (Z) for the development of severe (•), slight (□) and no damage (Δ) on red ginger inflorescences conducted in winter 1997. Equation represents the regression for slight damage symptoms only. Each data point represents mean from 5 to 8 inflorescences. Experiments were conducted separately due to flower availability.

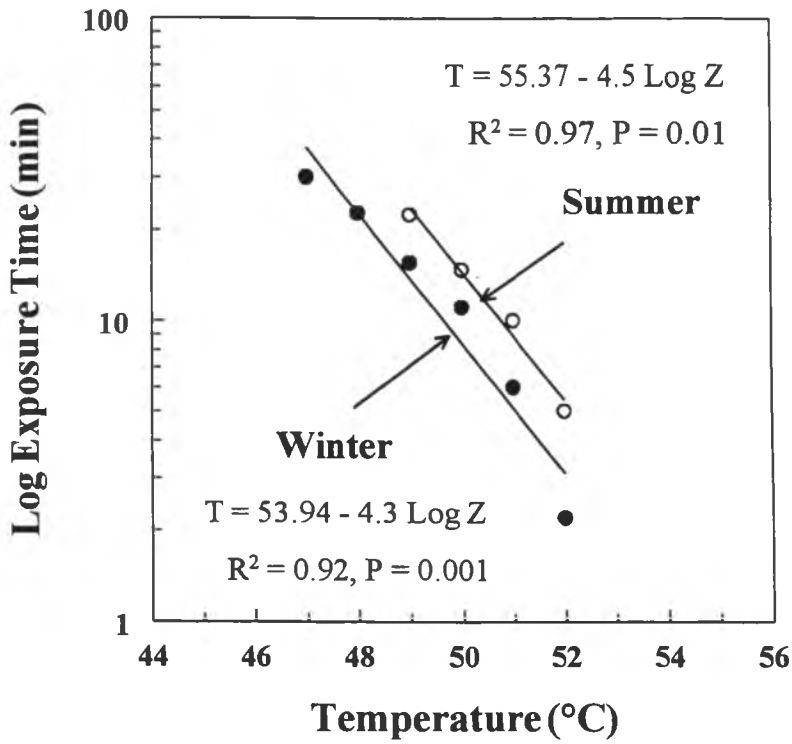


Figure 4.7. Comparison of threshold regression plots for slight damage symptoms for red ginger harvested in May to August (1995) and February to March (1997).

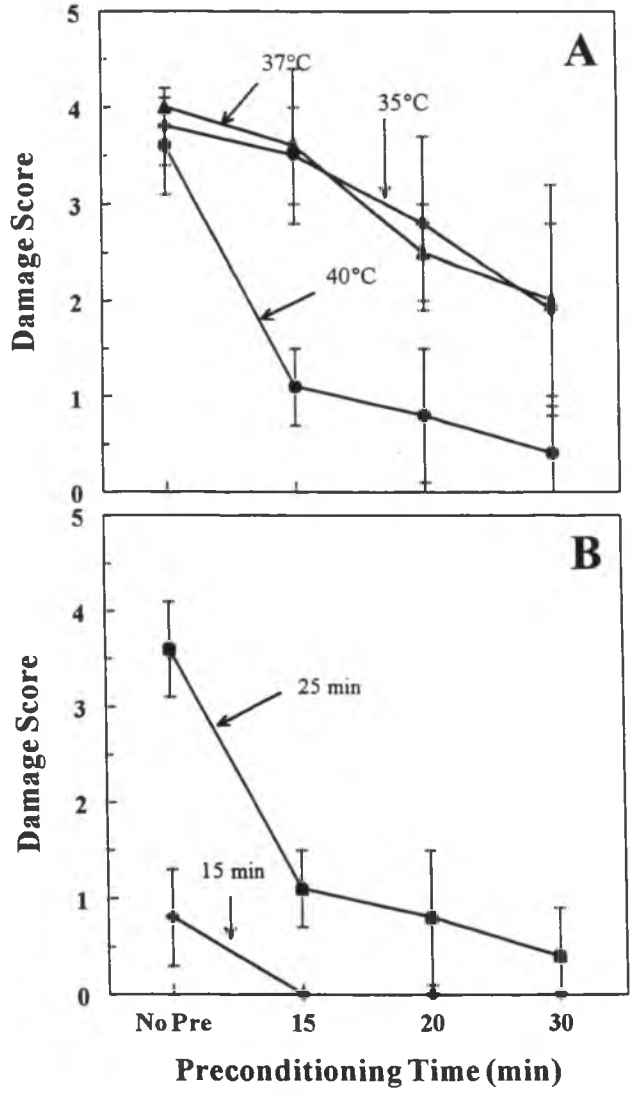


Figure 4.8. Thermotolerance induced by three different exposure times (15, 20, and 30 min) at three different preconditioning temperatures (35, 37.5, and 40°C) followed by hot water treatment at 50°C for 25 min (A), and by different exposure times at 40°C followed by hot water at 50°C for 15 and 25 min (B). Each data point represents mean from 10 inflorescences. Experiments were conducted separately due to inflorescence availability. Control (no preconditioning) treatments included in all experiments to allow comparison between experiments.

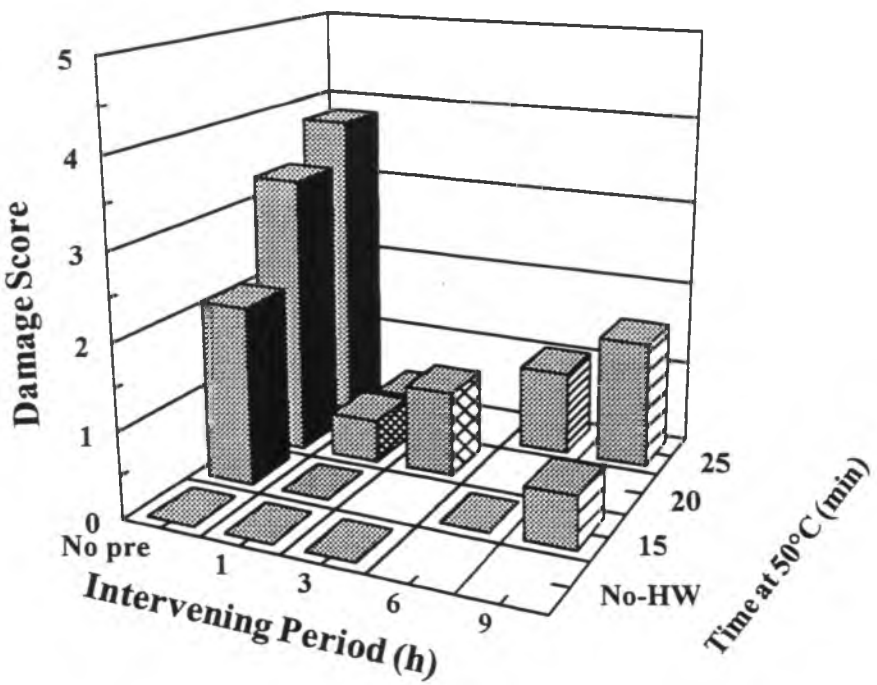


Figure 4.9. Intervening periods (1, 3, 6, and 9 h) between preconditioning induced-thermotolerance at 40°C for 30 min followed by hot water treatment at 50°C for 15, 20 and 25 min. Each data point represents mean from 10 inflorescences. Separate experiments were conducted for each interval between preconditioning treatment at (40°C for 30 min) and the hot water treatments (50°C for 15, 20 and 25 min) due to inflorescence availability. Control (no preconditioning) treatments included in all experiments to allow comparison between experiments.

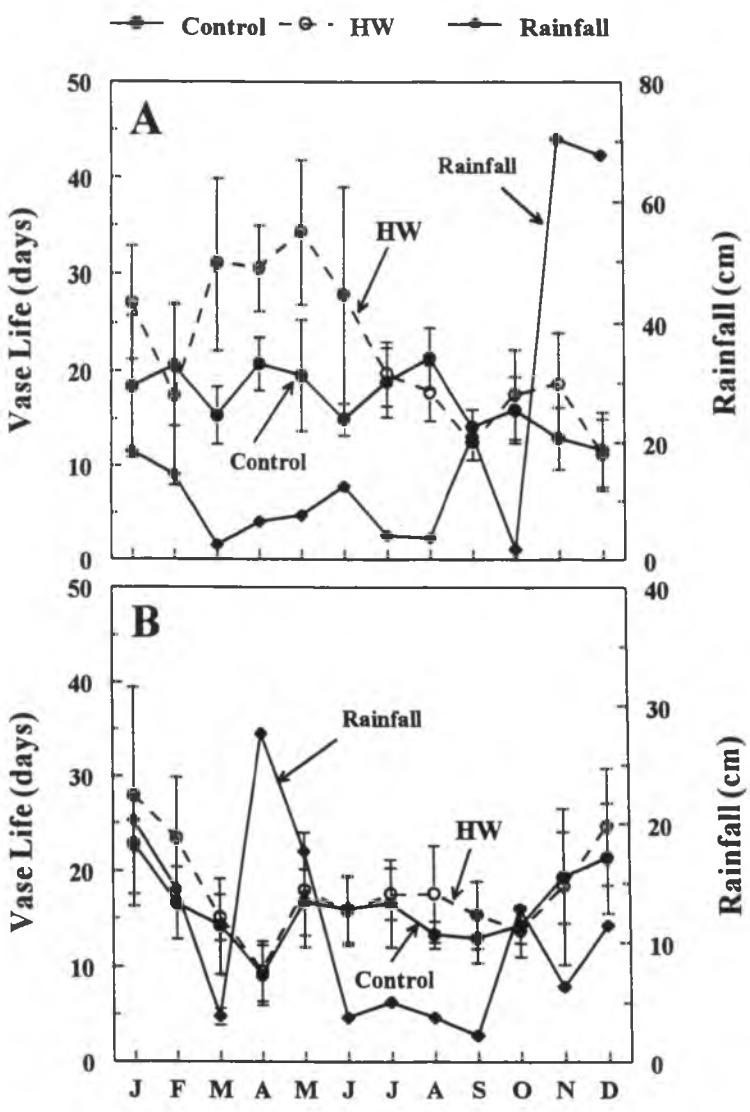


Figure 4.10. Postharvest vase life of unheated (•) and hot water treated (o) inflorescences, and amount of rainfall (♦) 30 days before harvest. Ten of 2/3 open stage inflorescences were harvested monthly up to 12 months for each treatment. Results show effect of hot water treatment (preconditioning at 40°C for 15 min, and then 50°C for 12 min) on vase life of two different cultivars: 'Red ginger' from Poamoho Experiment Station in 1996 (A) and 'Raspberry' from Waimanalo Experiment Station in 1997 (B).

CHAPTER 5

EFFECT OF HOT WATER ON POSTHARVEST PHYSIOLOGY AND GEOTROPIC RESPONSE

5.1. Introduction

Heat treatment by hot air or water has been used to extend postharvest vase life of several commodities such as papaya (Nishijima *et al.*, 1992), citrus (Rodov *et al.*, 1995), apple (Liu, 1978), tomato (Lurie and Klein, 1991), onion (Thamizharasi and Narasimham, 1993), broccoli (Forney, 1995), zucchini squash (Wang, 1994), and even cut flowers (Hara *et al.*, 1997; Hara *et al.*, 1996). Limited information is available regarding postharvest physiological changes for fruits and vegetables, and even less is known for cut flowers.

Ethylene production and respiration rate are major physiological factors involved in shelf life of several fruits, vegetables and some cut flowers (Kader, 1992). Several investigators have reported that heat treatment changes ethylene production and respiration rates. For example, heat treatment suppresses or delays the peak of ethylene production (Cheng *et al.*, 1988; Lurie and Klein, 1990; Lurie and Klein, 1991), whereas respiration rate increases, especially at the beginning of the treatment. However, respiration rate could be reversed or decreased after the fruit is removed from the stress treatments (Eaks, 1978; Inaba and Chachin, 1988). These changes in ethylene production and respiration rate, as mediated by heat treatment, can be used to determine postharvest life of the commodities. Effects of heat treatment on postharvest quality of fruits and

vegetables are also reported: apple fruit shows an increase in firmness and reduce titrable acidity (Liu, 1978; Lurie and Klein, 1992), and tomato shows reduced color development during ripening (Chang *et al.*, 1977).

Major factors possibly involved in red ginger senescence include respiration rate and sugar available to the cut inflorescence (Chapter 3). Other possible factors through which heat could have an effect include ethylene production, water balance of the cut stem, starch and sugar levels and microbial count. Investigation of these factors could provide a tentative mechanism as to how hot water increases red ginger vase life.

Hot water treatment has been shown to prevent geotropic response in red ginger inflorescence (Hara *et al.*, 1997) and asparagus (Paull and Chen, 1999). One thought proposes that geotropic response occurs when auxin is transported unequally between the upper and lower parts of the stem, or there is a change in tissue sensitivity to auxin (Salisbury, 1992; Salisbury, 1993; Salisbury *et al.*, 1988). Others involve in calcium movement as the accumulation of calcium prevents cell elongation (Friedman *et al.*, 1998). Since the accumulation of auxin in bending tissue precedes the increase in endogenous ethylene production, ethylene is thought to be one factor involving in geotropism (Wright *et al.*, 1978). Heat treatment could alter the balance of plant growth regulators or tissue sensitivity to auxin. Alteration of geotropic response may vary with inflorescence size and developmental stage. Included in this study was the effect of an auxin transport inhibitor and calcium chelator on red ginger geotropism.

5.2. Materials and methods

5.2.1. Effect of hot water on postharvest physiological factors

Inflorescences (cv. 'Red ginger') used in this study were obtained from a local grower in Waimanalo, on the Island of Oahu. Inflorescences were harvested and prepared as described in Chapter 2 (section 2.1) with the exception that the BA-treatment was replaced by the hot water treatment (precondition at 40°C for 15 min, and then 50°C for 12 min).

In general, after preparation, hot water was applied on the following day and was regarded as day 0 (one day after harvest). Determination of ethylene production and respiration rate was started a few hours after the treatment, and then every 24 h until the end of vase life. Water uptake and loss rates were determined daily. Counting of microorganisms and the extractions of sugar and starch content were conducted every 3 days until end of vase life. The methods to determine postharvest physiological factors were described in Chapter 2 and denoted by the section number in parenthesis: ethylene production and respiration rate (2.3.1), water balance of the cut stem (2.3.2), stem and vase microorganisms (2.3.3), sugar contents (2.3.4) and starch level (2.3.5). Experiments were performed twice and one experiment was selected to represent the result.

5.2.2. Effect of hot water on geotropic response

Inflorescences (cv. 'Red ginger') used in this study were harvested from Poamoho Experiment Station, on the Island of Oahu. Two-thirds opening stage inflorescences were harvested with stem length of 60 to 70 cm. After sorting for uniformity and the

stem was recut to the same length (55 cm) inflorescences were subjected to the hot water treatments (precondition at 40°C for 15 min and then hot water treatments). After the hot water treatments, stems were cooled in running water (22 ± 1°C) for 15 min. Control inflorescences were subjected to washing in tap water containing detergent for about the same time that hot water was applied. After treatments, all flower were recut to the same length (30 cm), and then leaves and leaf sheaths were removed. Inflorescences were held horizontally with the cut stem in wet floral foam (OASIS, Smithers-Oasis, Kent, OH, USA). The foam was kept wet by placing it in a tray containing 1,000 ml of DI water and water level was maintained at the same level throughout the study. Unless otherwise stated, small inflorescences with stem diameter ≤ 10.0 mm were used as they were easier to set into the floral foam. Inflorescences were held at 22 ± 2°C, 60 – 70% RH, and 12 h per day of fluorescent light (15 ± 4 $mmol \cdot m^{-2} \cdot s^{-1}$).

Geotropic curvature of the inflorescence tip from the main axis was measured daily for up to 7 days using a protractor. In a preliminary experiment, the inflorescence was set up in front of a digital camera (PDC 2000, Polaroid Corporation, Cambridge, MA) and photographs taken every 12 h for up to 7 days. After transferring the digital image to the computer, the angle of bending was analyzed using a computer program (Image Tool version 1.27, UTHSCSA, TX). The preliminary results indicated that the manual measurement with a protractor gave a similar result to that obtained with the digital camera. Subsequent experiments were, therefore, done using the protractor as the number of replications per treatment was more than could be done on the single digital camera.

Effect of different temperatures: Three different hot water temperatures (40, 45, and 50°C, for 15 min) were tested after preconditioning at 40°C for 15 min. Ten inflorescences were used per treatment (n = 10).

Effect of exposure times: Six different exposure times (0, 2.5, 5, 7.5, 10 and 12 min) were tested at 50°C. Five to eleven inflorescences were used per treatment. Experiments were conducted separately, due to inflorescence availability, and the results pooled for analysis.

Effect of inflorescence size: Two different size of inflorescences were also tested with the most effective treatments (50°C, 10 to 12 min): standard stem with diameter at the middle stem $\geq$ 10.0 mm (n = 6) and small stem with diameter $\leq$ 10.0 mm (n = 18) were compared.

Effect of developmental stages: Effect of hot water treatment (40°C for 15 min, then 50°C for 12 min) were tested on two different stages of inflorescence development were tested: two-third open (commercial stage, n = 9) and older stage (2 weeks after commercial stage, n = 7).

Effect of auxin transport inhibitor and calcium chelator: Only the inflorescences were dipped in ethylenediamine tetraacetic acid (Na-EDTA, 2.5 mM), a calcium-chelated compound, and 2,3,5-triiodobenzoic acid (TIBA, 200 ppm), an auxin transport inhibitor, for 30 min before being placed horizontally. Results were compared to the effect of hot water treatment (precondition at 40°C for 15 min, then 50°C for 12 min) and untreated control (submerge in tap water). Eight inflorescences were used per treatment (n = 8).

5.3 Results and discussion

5.3.1. Ethylene production and respiration rate

One mechanism of extending postharvest life is to suppress ethylene synthesis by heat treatment (Cheng *et al.*, 1988; Eaks, 1978; Inaba and Chachin, 1988; Lurie and Klein, 1990; Lurie and Klein, 1991). This suppression is a result of the inactivation of ACC synthase and ACC oxidase (Biggs *et al.*, 1988; Campbell *et al.*, 1997; Chan, 1986b; Paull and Chen, 1990; Yu *et al.*, 1980). However, in the red ginger system, ethylene has little effect on altering postharvest vase life (Fig. 3.4), and the production of ethylene by red ginger inflorescence was very low during inflorescence senescence (Fig. 3.5).

Ethylene production from hot water treated and unheated red ginger inflorescence was very low when compared to the respiration rate (Fig. 5.1A). There was no indication of a rise in ethylene production during red ginger senescence, although the patterns of change in ethylene production by senescence leaves does show a peak of ethylene production (Aharoni and Lieberman, 1979). Different responses may result from the difference in plant species and type of senescence symptoms. While Aharoni and Lieberman (1979) suggested that the rise of ethylene production during senescence could be subsequently involved in inducing leaf abscission, the modified leaves or bracts of red ginger do not show abscission during senescence.

The respiration rate of hot water treated inflorescences was significantly lower a few days after treatment than unheated inflorescence ($P = 0.0001$), although the rate of respiration was higher during the first day after treatment (Fig. 5.1A). The effect of heat treatment on increasing respiration rate has also been reported for apple during storage at

38°C (Lurie and Klein, 1990), tomatoes at 36, 38 or 40°C (Lurie and Klein, 1991), and avocado at 30 and 35°C (Eaks, 1978; Inaba and Chachin, 1989). However, once fruit is returned to ambient temperature (20°C), the respiration rate of treated fruit is lower than untreated fruit. The pattern of change in respiration rate of hot water-treated red ginger was similar to that of heat-treated fruit (Eaks, 1978; Lurie and Klein, 1990; Lurie and Klein, 1991). The initial increase in the respiration rate of red ginger inflorescence after heat treatment may be due to the plant response to heat stress associated with changing mitochondrial activity. In avocado, the respiration rate increases with increasing storage temperatures from 25 to 35°C and the inhibition of mitochondrial oxidation by cyanide decreases (Inaba and Chachin, 1989) indicating the development of cyanide-insensitive respiration during heat stress. The lower respiration rate of hot water treated inflorescences than unheated inflorescences (Fig. 5.1A), suggested the relationship between respiratory substrate and inflorescence vase life.

5.3.2. Water balance of the cut stem

Postharvest changes of water uptake and water loss rate were similar to that in the previous study (Fig. 3.6). Water uptake and water loss both decreased with time after harvest ($P = 0.0001$), and there was no significant difference between hot water treated and unheated inflorescences (Fig. 5.2A). During the first 4 days after treatment, the rate of water uptake and water loss rate of hot water treated inflorescences were higher than that of the control. Later, the water uptake and water loss of hot water treated and unheated inflorescences were similar until the unheated inflorescences started to senesce.

Once senescence started, ca. 8 to 10 days for unheated inflorescence and 13 to 14 days for hot water treated inflorescence, the rate of water loss was higher than water uptake.

After harvest, water uptake and water loss occurred at similar rates resulting in a stable water balance until the onset of inflorescence senescence (Fig. 5.2B). Once inflorescence started to senesce, water loss exceeded water uptake, and a negative water balance occurred. Senescence symptoms increased as inflorescence water balance became increasingly negative (Fig. 5.2B). It was not clear which was the cause or effect as the senescence symptoms and negative water balance occurred simultaneously.

5.3.3. Stem and vase microorganisms

Total microbial count for stem segments increased with time up to 15 days after harvest, and then decreased in hot water treated stem (Fig. 5.3A). Due to high variation in microbial count among the samples, no significant difference was found between hot water treated and unheated stems. The microbial count in the vase solution tended to increase with time after harvest (Fig. 5.3B). Although no statistically significant difference was found between unheated and hot water treated inflorescence for microbial counts in both stem segments and vase solutions, senescence symptoms developed earlier (9 days) in unheated inflorescences than hot water treated inflorescences (15 days) (Fig. 5.3C).

Although hot water or heat treatments have been reported to control fungal disease in fruit such as apple (Klein *et al.*, 1997) and banana (Reyes *et al.*, 1998), the use of heat treatment to control bacterial growth as postharvest treatment is rare. Hot water

treated-stems tended to have lower microbial counts compared to unheated stems (Fig. 5.3A; B). However, the temperature and exposure time used in our study (50°C, for 12 min), has not been shown to control microbial growth (Lurie, 1998).

5.3.4. Levels of total sugar and sucrose

Total sugar in inflorescence bracts was maintained at a high level for the first few days, then declined after harvest ($P = 0.0001$) for both unheated and hot water treated inflorescences (Fig. 5.4A). Total sugar was maintained at a high level, for longer time, in hot water treated bracts than unheated bracts ($P = 0.0001$). The decline in bract total sugar (Fig. 5.4A) occurred at approximately the same time as the senescence rating started to increase (Fig. 5.3C). This finding suggested that the longer high sugar content was maintained in the bracts, the longer the vase life. In contrast to the levels of total sugar in the bract, stem total sugar significantly declined after harvest ($P = 0.0001$, Fig. 5.4B) with the levels of sugar remaining being significantly higher in hot water treated than unheated stems ($P = 0.0001$). The total sugar on day 12 (last day of measurement for control inflorescences) was about 44% (unheated) and 62% (HW treated) of the original levels in the stems compared to the values of 76% (unheated) and 108% (HW-treated) in the bracts. The sharp reduction in stem sugars may have resulted from the demand for sugar by the inflorescence bract tissues, acting as an active metabolic sink (Ho and Nichols, 1977).

The change in bract and stem sucrose was similar to that of the total sugar in bract and stem (Fig. 5.4C and D), as the sucrose level declined after harvest ($P = 0.0001$).

Nine days after treatment, hot water treated inflorescences had significantly higher level of sucrose than unheated inflorescences for both bracts ($P = 0.04$) and stems ($P = 0.0001$) and this relationship persisted petal senescence. Hot water may extend red ginger vase life by maintaining a high level of endogenous sugar in the stems, and subsequently in the bracts, but how hot water maintained these high levels of endogenous sugar in the bract tissues was not determined.

Heat treatment does not increase sugar content in all plant species. Storing apples at 38°C for 4 days has little effect on level of total soluble solids content (Lurie and Klein, 1990), while muskmelons treated at 45°C for 3 h then kept at 4°C for 18 days have higher reducing sugar level than unheated fruits (Lingle *et al.*, 1987). These results suggested that the responses of plant tissues to heat treatment vary with plant species.

5.3.5. Starch level

Starch level in bracts remained unchanged throughout aging, whereas that of the stem decreased with time after harvest ($P = 0.0001$, Fig. 5.5). However, there was no significant difference between unheated and hot water treated stems. This finding suggested that starch degradation could not account for the change in sugar levels mediated by hot water treatment. A similar effect was reported for apple as Lurie and Klein (1990) who concluded that starch breakdown is not enhanced by heat treatment and that sugar content is not affected by the treatment of apples at 38°C for 4 days.

5.3.6. Effect of hot water on geotropic response

Hot water treatment can minimize the negative geotropism in red ginger inflorescence (Hara *et al.*, 1997) and asparagus (Paull and Chen, 1999). In control inflorescences, the geotropic response could be seen within 6 h of placing the inflorescence horizontally. In snapdragon, negative geotropism recorded by a video camera revealed that the response of the inflorescence to gravitational pull occurred within seconds (Reid, 1999, personal communication). In red ginger, tips of unheated inflorescences curved almost 90° from the original position within 3 days, whereas hot water treated inflorescences showed no sign of negative geotropic response (Fig. 5.6). The result showed that hot water was a promising treatment to control geotropic response in red ginger and possibly other inflorescences, such as gladiolus and snapdragons.

Effect of different temperatures: Although wide ranges of hot water temperatures are effective in controlling insect pests and decays (Couey and Hayes, 1986; Hansen and Hara, 1994; Hansen *et al.*, 1992; Hara *et al.*, 1996; Reyes *et al.*, 1998), only a narrow range of temperature was effective in preventing negative geotropic response in red ginger. Dipping inflorescence 15 min in hot water at 40°C did not prevent geotropism compared to 45 and 50°C (P = 0.0002, Fig. 5.7). Hot water at 45°C delayed the geotropic response for about 3 days after which the geotropic response resumed (Fig. 5.7A). Hot water at 50°C completely suppressed geotropic response for, at least 7 days (Fig. 5.7B). This finding suggested a temperature sensitivity to the gravity sensing mechanism.

Effect of exposure times: The longer the exposure time at 50°C, the longer the period that hot water suppressed the negative geotropic response (Fig. 5.8A). The 10 min exposure completely controlled the geotropic response for at least 7 days (P = 0.0001, Fig. 5.8B). This finding suggested that when applying hot water at 50°C, at least 10 minutes was required in order to prevent the geotropic response in red ginger inflorescence. The short period of geotropism suppression at shorter exposure times (shorter than 10 min at 50°C) suggested that inflorescences recovered from the heat treatment, known as a repair mechanism. The repair mechanism occurred when the stress treatment did not exceed the damage threshold, and the plant resumed normal metabolism as before the heat treatment was applied (Inaba and Chachin, 1988).

Effect of inflorescence size: Hot water treatment at 50°C significantly suppressed geotropic response in red ginger inflorescences compared to unheated inflorescences when the exposure time was 12 min (P = 0.0001, Fig. 5.9A; B). At 10 min-exposure, the hot water at 50°C controlled geotropism more effectively in the smaller stem diameter inflorescence than the larger stem diameter inflorescence (P = 0.03). The smaller diameter inflorescences showed a trend for greater curvature angle with time (Fig. 5.9C), but the increase was not significantly different from the first day of measuring. By comparison, larger diameter stems resumed geotropic curvature after 4 days (Fig. 5.9D). These results suggested that hot water at 50°C for 12 min was insufficient to suppress the geotropic response in the large inflorescences, and they may recovered from the heat stress after 4 days of heat removal. There was an interaction between stem diameter and exposure time at 50°C (P = 0.03); the larger the stem diameter, the longer the exposure

time required to prevent negative geotropic curvature in red ginger inflorescences. These findings suggested that effective treatment, as the relationship between the hot water temperature and exposure time, to control geotropic response in red ginger inflorescences depended on the inflorescence size that was submerged to the hot water tank.

Effect of developmental stage: Older inflorescences showed less curvature than less open inflorescence (Fig. 5.10A; B). Hot water at 50°C for 12 min completely controlled geotropic response in both stages of development for up to 7 days (P = 0.0001). Curvature of the older inflorescence took place only at the tip of inflorescence (about ¼ of the inflorescence), whereas a less open stage ($^2/_3$ open) started to curve at about ½ of the inflorescence length. This difference in location where the curvature took place may reflect lignification of the rachis as reported in older stems of cut rose (Parups and Voisey, 1976). However, the definition of inflorescence developmental stage and the lignification of inflorescence stem may vary with plant species, as less mature snapdragons show less geotropic bending than the older stages (Teas and Sheehan, 1957).

Effect of auxin transport inhibitor and calcium chelator: Asymmetric lateral movement of auxin is thought to be major factor accounting for plant tropism based on Cholodny-Went theory (Harrison and Pickard, 1989; MacDonald and Hart, 1987), although controversy still exists (Trewavas, 1992a; Trewavas, 1992b). Despite the controversy in Cholodny-Went theory, auxin is indeed essential for the cell elongation that causes bending of the tissue and the removal of auxin prevents curvature (Salisbury, 1993). In the opposite direction of auxin movement and function, the accumulation of calcium restricts tissue elongation at the site of accumulation, therefore, the curvature

takes place due to unequal elongation of the stem (Friedman *et al.*, 1998; Philosoph-Hadas *et al.*, 1996).

The auxin transport inhibitor (TIBA) and calcium chelator (EDTA) on red ginger geotropism were compared to the effect of the hot water treatment (50°C, for 12 min). Dipping the inflorescence in 200 ppm TIBA for 30 min gave essentially the same response as the hot water treatment in preventing geotropic curvature when compared to the unheated and EDTA treated inflorescences ($P = 0.0001$, Fig. 5.11A; B). The failure to prevent geotropic curvature by dipping the inflorescence 30 min in 2.5 mM EDTA does not rule out an effect of calcium in red ginger geotropism as the concentration may have been too low, application method inadequate, or the calcium chelator was ineffective (Friedman *et al.*, 1998). These findings suggested that auxin was involved in red ginger geotropism, and that hot water may, therefore, disrupt either auxin synthesis, transport, perception, or response.

5.4. Summary

Selective hot water treatment at 50°C for 12 min (preconditioned with 40°C for 15 min) extended red ginger vase life and initially increased the respiration rate which then declined to a rate less than unheated inflorescences ($P = 0.0001$). Hot water had little or no effect on ethylene production, starch metabolism or the total number of microorganisms in the stem sections and the vase solution. While hot water treatment helped to maintain the water balance of the cut inflorescence stem, the mechanism was not clear. A cause-effect relationship was not found between water balance and

inflorescence senescence for red ginger. Hot water treated inflorescences showed a trend to maintain higher sugar levels than unheated inflorescence and consequently, the inflorescence lasted 5 to 6 days longer than unheated inflorescences. Hot water also suppressed the negative geotropic response in red ginger inflorescences. The relationship between temperature, exposure time and inflorescence size were important in modifying the geotropic response. Treatment of inflorescences with auxin inhibitor (2,3,5-triiodobenzoic acid) suggested that auxin was involved in red ginger geotropism, and hot water may disrupt either auxin synthesis, transport, perception, or response.

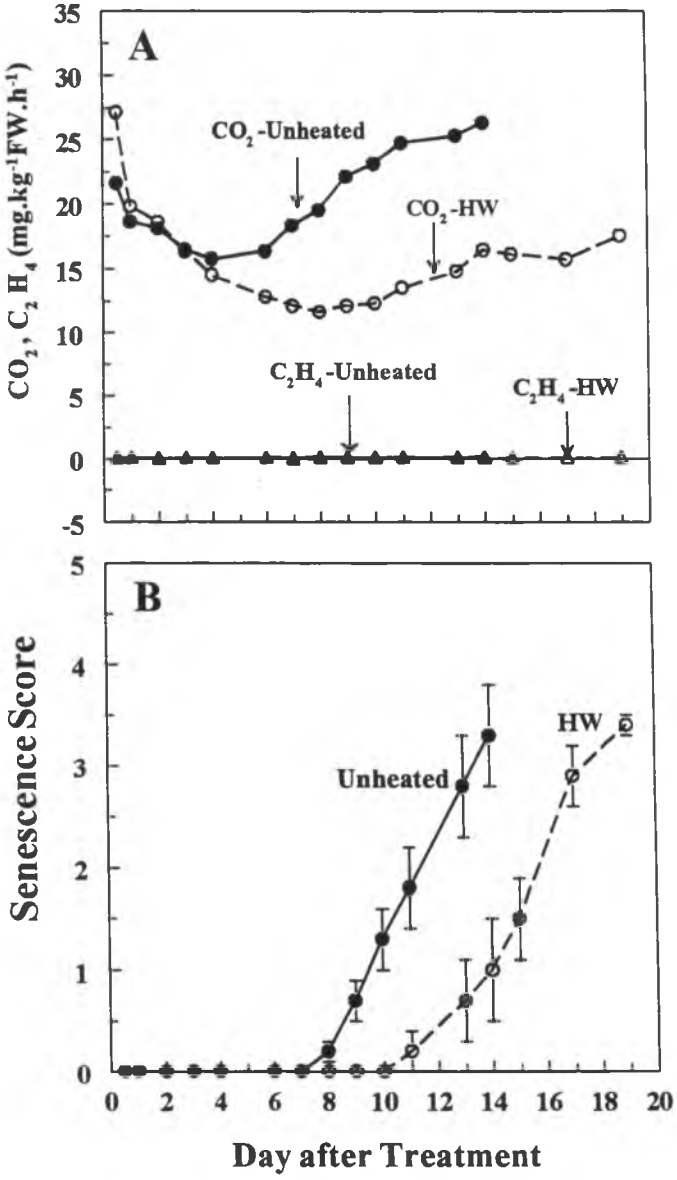


Figure 5.1. Changes in ethylene production and respiration rate (A), and senescence score (B) in unheated (•) and hot water treated (o) inflorescences. Data were collected daily from few hours after treatment (Day 0) until end each treatment vase life. Each data point represents mean from 3 respiratory chambers. Experiments were performed twice and one experiment was selected to represent the results.

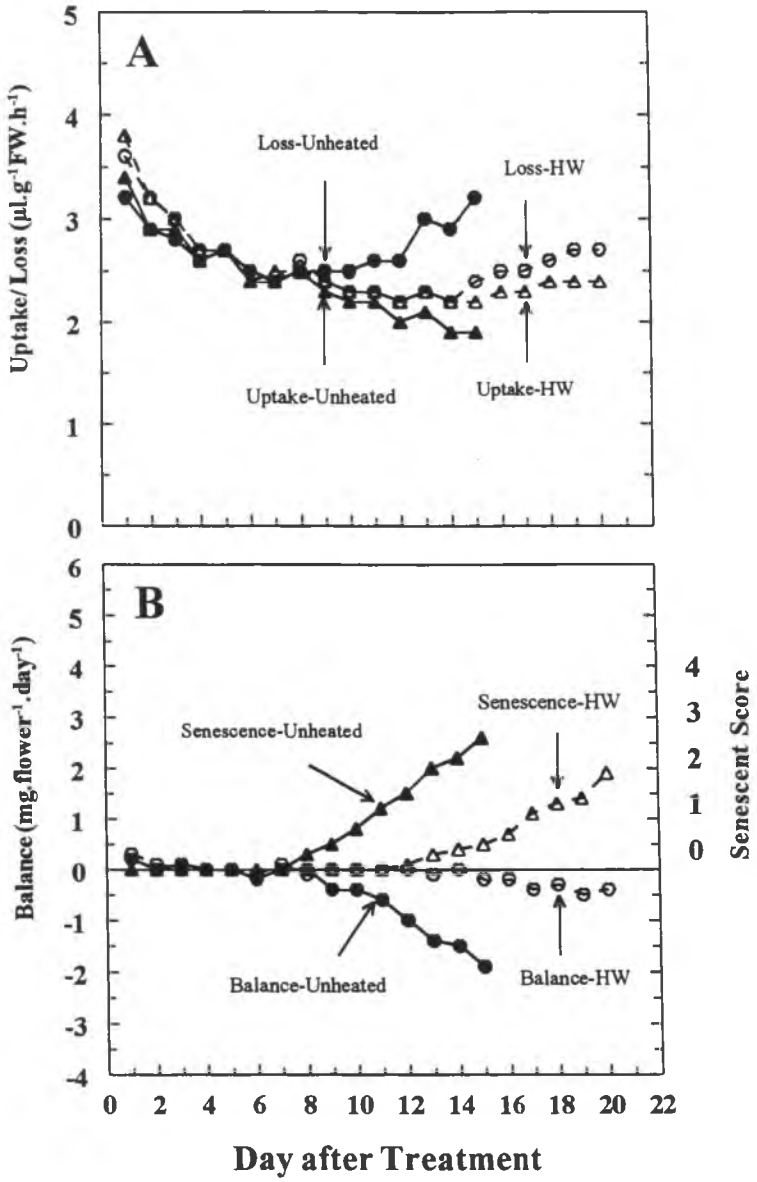


Figure 5.2. Changes in water uptake and water loss rate (A), and water balance and senescence score (B) in unheated (•) and hot water treated (o) inflorescences. Data were collected daily from one day after treatment until end of each treatment vase life. Each data point represents mean from 10 inflorescences. Experiments were performed twice and one experiment was selected to represent the results.

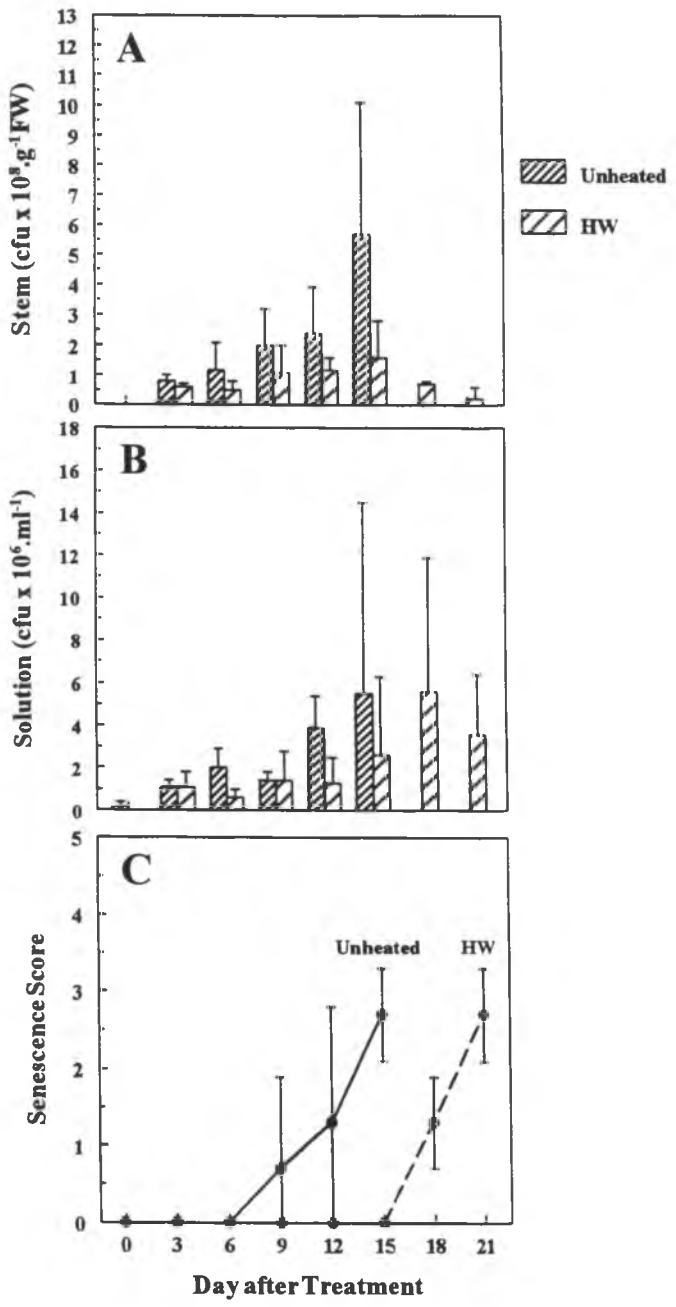


Figure 5.3. Total count microorganisms in the stem segments (A) the vase solution (B) and senescence score (C) in unheated (•) and hot water treated (o) inflorescences. Samples were taken after experimental set up (Day 0), and 3, 6, 9, 12, and 15 after treatment for unheated inflorescences, and up to 18, and 21 days for hot water treated inflorescences. For each sampling date, 3 stems were used per treatment. Experiments were performed twice, and one experiment was selected to represent the results.

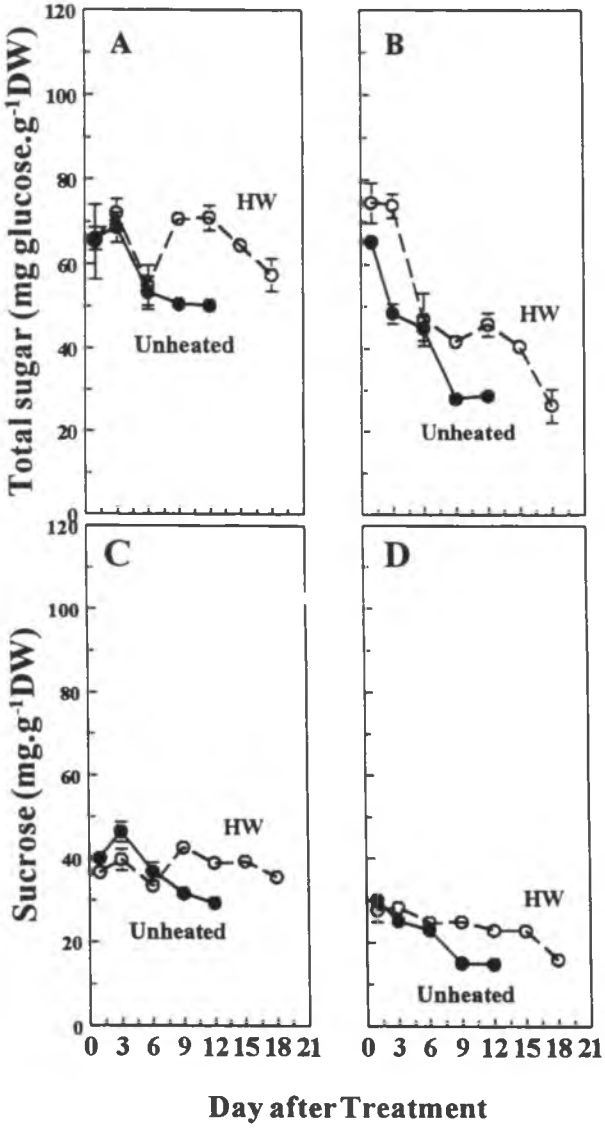


Figure 5.4. Total ethanol soluble sugar in bracts (A) and in stem (B), and sucrose in bract (C) and in stem (D) in different inflorescence parts of unheated (•) and hot water treated (o) inflorescences. Sugar extractions were performed on 1, 3, 6, 9, 12 days after treatment for unheated inflorescences, and up to 15, and 18 days for hot water treated inflorescences. For each sampling date, samples were combined from 3 inflorescences and sugar extractions conducted separately 3 times. Experiments were performed twice, and one experiment was selected to represent the results.

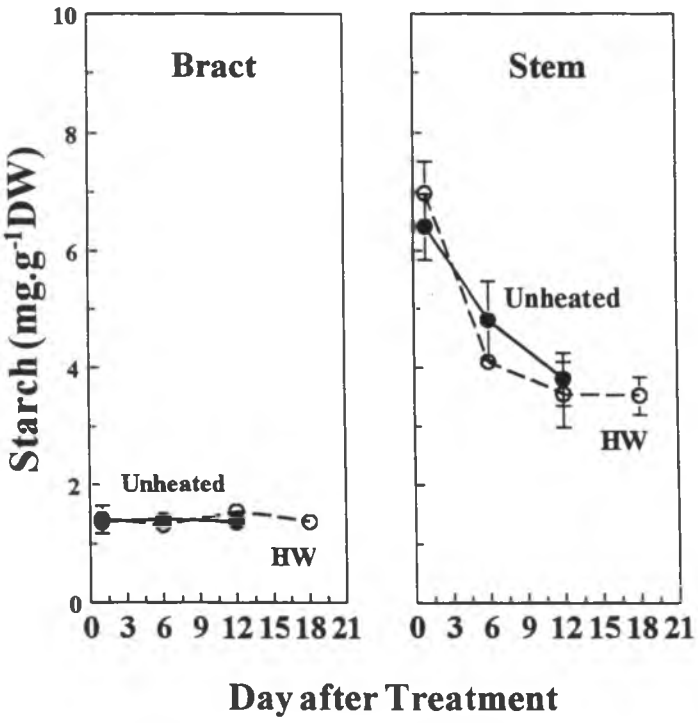


Figure 5.5. Changes in the starch content of different inflorescence parts of unheated (•) and hot water treated (o) inflorescences. Starch extractions were performed on 1, 6, 12 days after treatment for unheated inflorescences, and up to 18 days for hot water treated inflorescences. For each sampling date, samples were combined from 3 inflorescences and starch extractions performed separately 3 times. Experiments were performed twice, and one experiment was selected to represent the results.

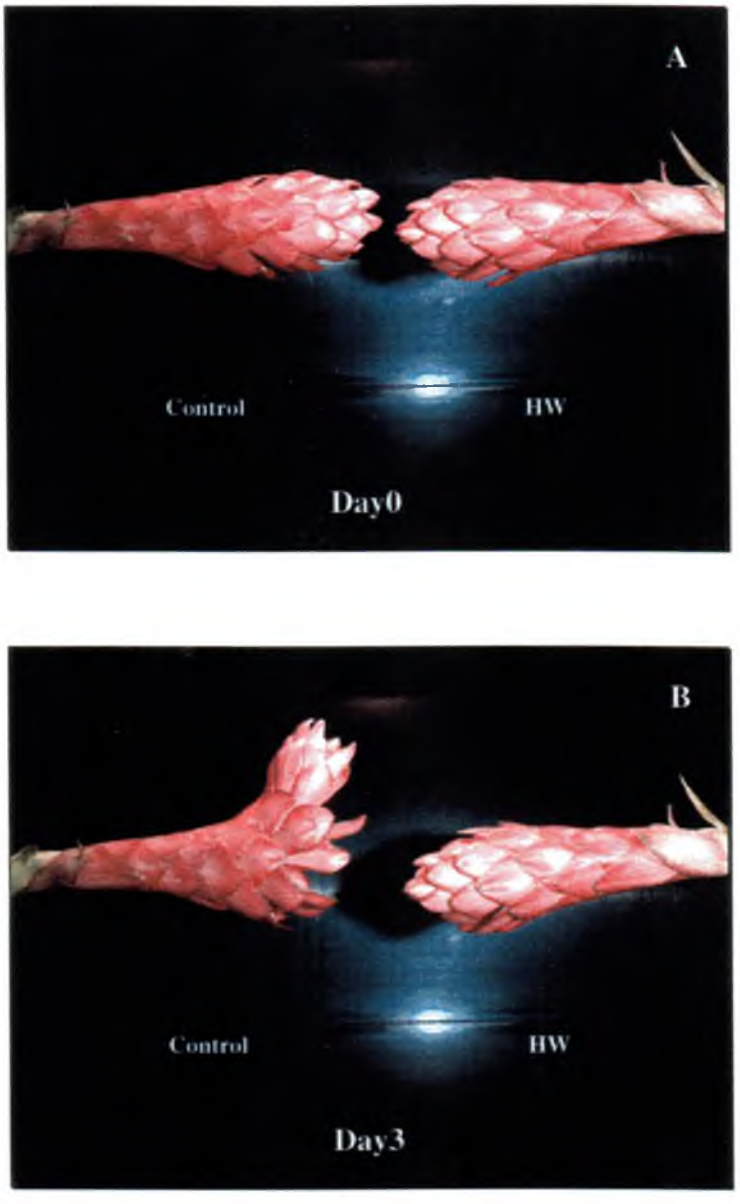


Figure 5.6. Effect of hot water (50°C, 12 min) on controlling geotropic curvature in red ginger inflorescences. Data were compared from whether the inflorescences were set horizontally (Day 0, A) and the three day later (Day 3, B).

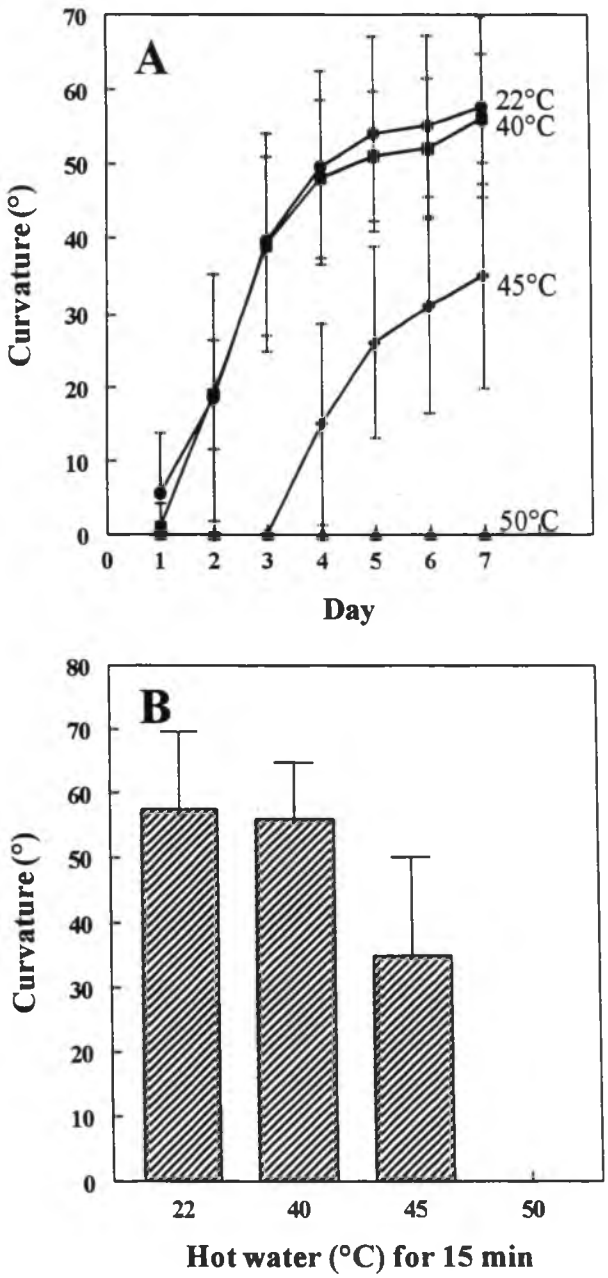


Figure 5.7. Degree of curvature (A) and the curvature at 7 days (B) of the red ginger inflorescences treated for 15 min at 40, 45, and 50°C. All hot water treatments were applied after preconditioning treatment at 40°C for 15 min. Each data point represents the mean from 10 inflorescences.

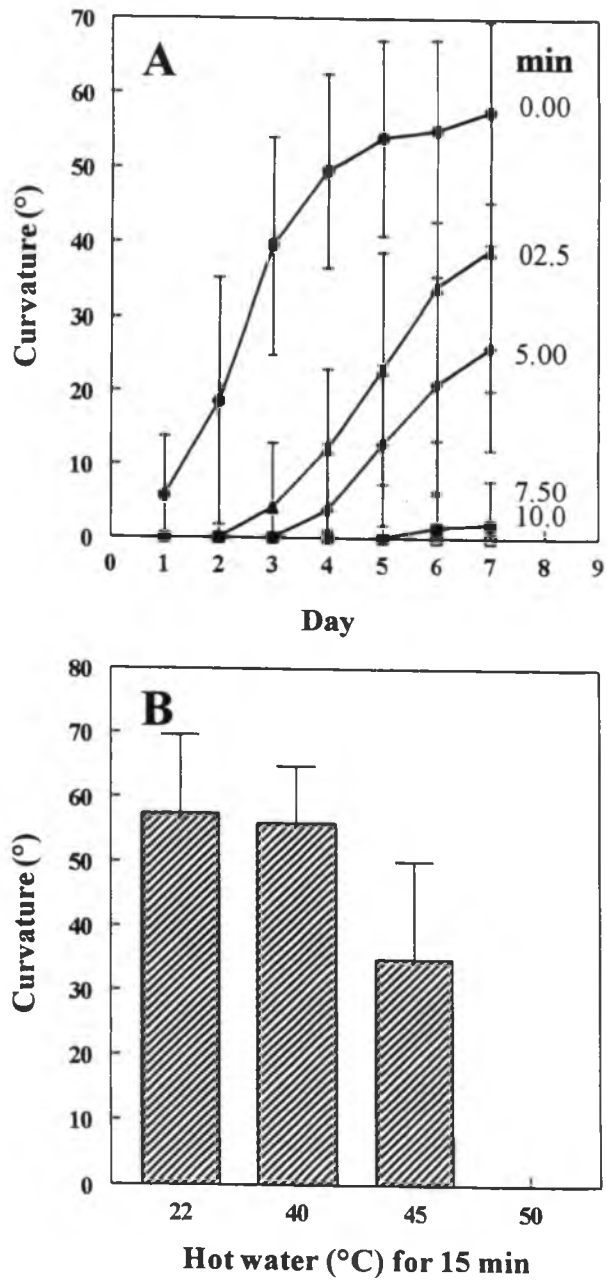


Figure 5.8. Degree of curvature (A) and the curvature at 7 days (B) of the red ginger inflorescences treated at 50°C for 0, 2.5, 5, 7.5, and 10 min. All hot water treatments were applied after preconditioning treatment at 40°C for 15 min. Each data point represents the mean from 10 inflorescences.

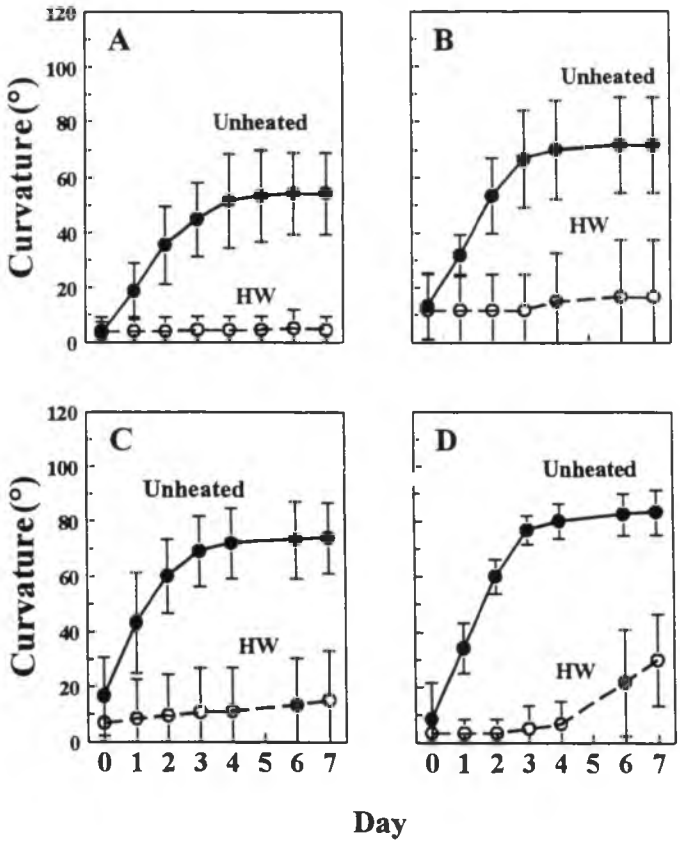


Figure 5.9. Effect of stem size: small (diameter at middle stem ≤ 10.0 mm, A, C) and large (diameter at middle stem ≥ 10.0 mm, B, D) stems, and two exposure times at 50°C: 12 min (A, B) and 10 min (C, D) on the development of geotropic curvature. Experiments were compared between unheated (•, no hot water treatment) and hot water treated (o, at 40°C for 15 min, then at 50°C for assigned times) inflorescences. Each data point represents the mean from 6 inflorescences for large stems, and 18 inflorescences for small stems.

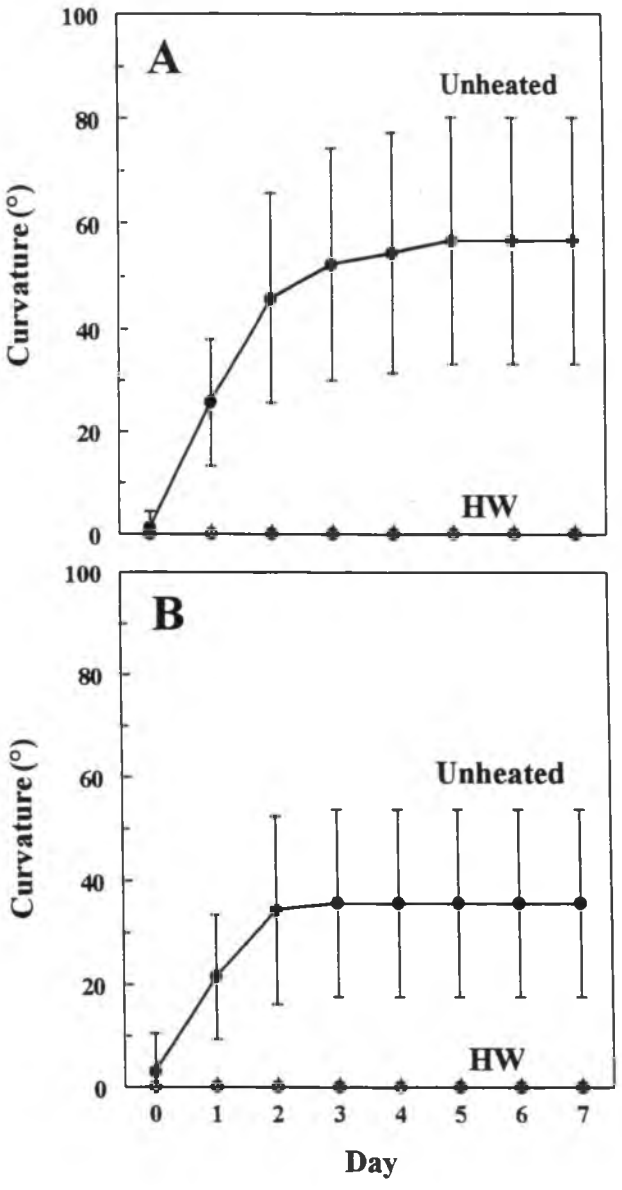


Figure 5.10. Effect of two developmental stages: 2/3 open (A) and fully open (B) on geotropic curvature of red ginger inflorescences. Experiments were compared between unheated (•, no hot water treatment) and hot water treated (o, at 40°C for 15 min, then at 50°C for 12 min) inflorescences. Each data point represents the mean from 9 inflorescences.

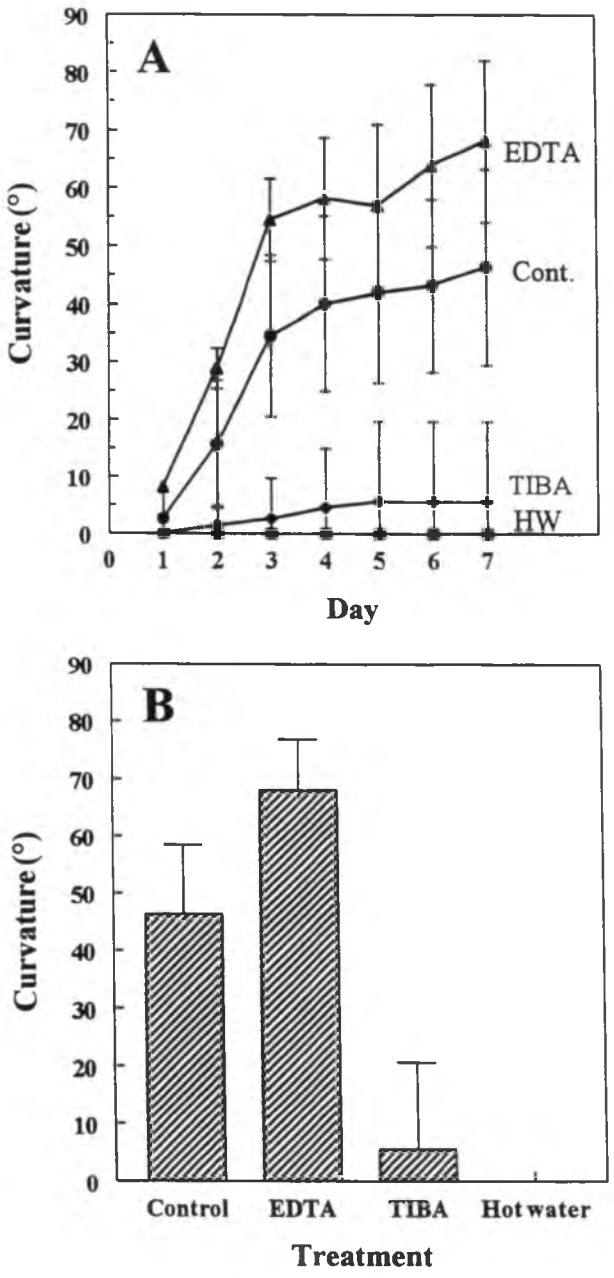


Figure 5.11. Degree of curvature (A) and the curvature at 7 days (B) of the red ginger inflorescences treated with calcium chelator (Na-EDTA, 2.5 mM), auxin transport inhibitor (TIBA, 200 ppm) and hot water treatment (40°C for 15 min and then at 50°C for 12 min). Chemical treatments were applied by submerging only the inflorescences into the solutions for 30 min. Each data point represents the mean from 10 inflorescences.

CHAPTER 6

EFFECT OF HOT WATER ON ENERGY RESERVES AND ENZYME ACTIVITY

6.1. Introduction

Previous studies have shown that a hot water treatment helped to maintain a high level of sugar content in plant tissues compared to unheated inflorescence. It is believed that, because of the higher sugar content in the inflorescence bracts and stems, the inflorescence lasted longer.

Sucrose in bleeding sap from maize stems was estimated to be only 0.04% of the sap volume (Canny and McCully, 1988) suggesting that the major sugar in the stem section may be located in the plant tissues (symplast). Sugar contents in different tissues of red ginger have not been determined. In cut rose after harvest, leaves and stems supply the flower buds with carbohydrate, and the extent of the energy reserves is correlated with vase life: the higher the carbohydrate reserves, the longer the flower vase life (Marissen and Brijn, 1995). In red ginger, this information is not available.

Sugar content of leaves of *Dactylis glomerata* increases after a short period of exposure (10 second) at 50°C (Zavadskaya, 1963). The increase in sugar content of plant tissues after heat shock may result from a protective mechanism in which sugar helps to prevent the denaturation of plant proteins (Belikov *et al.*, 1963). The mechanism as to how sugar content increases or is maintained at a higher level after heat treatment has not been investigated. However, it has been suggested that some of the processes responsible

for sucrose degradation may be heat sensitive (Lingle *et al.*, 1987). The sucrose degradation mechanism involves the activity of the sucrose metabolic enzymes: sucrose phosphate synthase (SPS), sucrose synthase (SS), and invertase (Kruger, 1990).

The objectives for these studies, therefore, were to determine the sugar content in different compartments (apoplast and symplast), the sugar content in different parts of the inflorescence, and the effect of hot water treatment on sugar content and sugar metabolic enzymes. Results from these studies should lead to an understanding of the mechanism by which hot water helps to maintain a higher level of sugar content in the inflorescences, and consequently, extend vase life.

6.2. Materials and methods

Red ginger inflorescences (cv. 'Red ginger') used in this study were harvested (70-75 cm) from plants grown at Poamoho Experiment Station on the Island of Oahu. Inflorescences were harvested at the two-thirds open stage in the morning and transported in water to the laboratory within 3 h. After culling for uniformity, stems (with one leaf) were cut to the same length (55 cm). The unheated control inflorescences were washed with 7.5 $g \cdot l^{-1}$ Liqui-Nox detergent (Alconox Inc., N. Y.) to remove aphids and other insects. In general, the preparation of the plant materials including washing of the unheated inflorescences was done in the afternoon. The hot water treatment (precondition 40°C for 15 min, then 50°C for 12 min) were applied on the following day (one day after harvest). Protocol used to apply hot water treatment was as previously described in section 4.2.2 by bunching inflorescences into 10 and submerging 2 bunches

in hot water tank at a time. The dryness of the washed materials and the hot water treated inflorescences was carried out at room temperature (23 ± 2°C) by allowing the inflorescences to stand in buckets containing tap water for 6 to 12 h before being used as starting materials.

6.2.1. Apoplastic and symplastic sugars

Centrifugation was used to determine the sugar content of the apoplastic and symplastic tissues. This was a modification of the procedure developed for sugarcane leaves (Meinzer and Moore, 1988), strawberry (Pomper and Breen, 1995), and pea epicotyl (Terry and Bonner, 1980).

Pea epicotyl subjected to 3,000 x g shows significant cell damage (Terry and Bonner, 1980). Preliminary study with red ginger showed that the accumulation of sap in a 1.5 ml Eppendorf tube increased with increasing centrifugal force (g) up to 1,310 g (4,000 rpm) for 15 min (Fig. 6.1A). Force up to 2,040 x g (5,000 rpm) did not further increase the volume of sap collected from the stem section. However, at forces greater than 2,040 x g, (i.e., 2,940 x g or 6,000 rpm) the volume of sap collected increased. The increase in collected sap beyond 2,040 x g may be associated with symplast contamination due to cell damage, as reported by Terry and Bonner (1980). To determine the centrifugation time, a force of 2,040 x g (5,000 rpm) was used, collected sap increased up to 8 min. There was no further increase in collected sap at a time longer than 8 min (Fig. 6.1B). Therefore, to separate apoplast (sap) from symplast (tissue), stem

sections were routinely centrifugation at 2,040 x g for 10 min to assure that sap was completely removed.

Stem section (2.5 cm long) from various locations was cut longitudinally with a 0.8 cm diameter cork border. The sections were trimmed from both ends to a final length of 1.5 cm. Contamination of symplast from cut surface was removed by dipping the stem section in two different beakers, containing 300-ml DI water, for 10 second each. After being blotted dry, fresh weight was taken before being placed in the Eppendorf tube (initial weight) and kept on ice before centrifugation. Based on preliminary results, centrifugation was carried out at 2,040 x g (5,000 rpm, Standard rotor, Eppendorf microcentrifuge 5415C, Brinkmann Instrument, Co., Westbury, N. Y.) for 10 min. Sap volume was determined as the weight difference between Eppendorf tubes (+ Sap) with and without the stem section. After all weight and volume were determined, the sap was frozen in liquid nitrogen for sugar determination. After centrifugation, the stem sections were frozen in liquid nitrogen for sugar extraction on the same day as the centrifugation, and sugar content determined the following day.

For each treatment and sampling day, a total of 12 sections (ca. 2-3 cm each section) were cut from 3 different stems. For each stem, four sections were cut from various locations assigned as followed:

Section 1, base of stem, cut 30 cm from the base of inflorescence (Stem –1)

Section 2, middle of stem, cut 15 cm from the base of inflorescence (Stem-2)

Section 3, top of stem, cut 2 cm from the base of inflorescence (Stem-3)

Section 4, rachis, cut the middle length of the rachis (Rachis)

Each section was centrifuged, sugar content determined, and the mean represent for 12 sections (n = 12), comparing between unheated control and hot water treated inflorescences. Samples were taken at 1, 5, 10, and 18 days after harvest.

The extraction of sugar from frozen tissues was as previously described in section 2.3.4 with some modification. After centrifugation, the whole frozen stem section (ca. 280 mg) was carefully ground to powder in the presence of liquid nitrogen and 10 ml of 90% ethanol was added. After homogenizing for 15 seconds, the tissue homogenate was centrifuged at 11,672 x g (10,000 rpm, SA 600 rotor, Sorvall RC-5B centrifuge, Du Pont Instruments, Newtown, CT) at 4°C for 20 minutes. Clear supernatant was transferred to capped bottles and kept at 4°C until used to determine total ethanol soluble sugar and sucrose.

Total ethanol soluble sugar content and sucrose were determined by phenol-sulfuric acid method (Dubois *et al.*, 1956), and in the presence of 30% KOH (van Handel, 1968) for sucrose determination. The level of reducing sugar was indirectly determined by subtracting the sugar level with and without the presence of 30% KOH. Detailed procedures for sugar determination were previously described in section 2.3.4 and summarized in Appendix C.

6.2.2. Sugars in different parts of inflorescence

To determine sugar in various plant parts, inflorescence stem (40 cm long) was separated into 8 sections as follows:

Section 1, bract 1, top portion of the inflorescence (Bract-top)

Section 2, rachis 1, top portion of the inflorescence (Rachis-top)

Section 3, bract 2, bottom portion of the inflorescence (Bract-bot)

Section 4, rachis 2, bottom portion of the inflorescence (Rachis-bot)

Section 5, stem 1, cut 0 to10 cm below the inflorescence (Stem-10)

Section 6, stem 2, cut 10 to 20 cm below the inflorescence (Stem-20)

Section 7, stem 3, cut 20 to 30 cm below the inflorescence (Stem-30)

Section 8, stem 4, cut 30 to 40 cm below the inflorescence (Stem-40)

The levels of sugar were compared between unheated and hot water treated inflorescences harvested one week apart from each other, due to the inflorescence availability. Inflorescences were sampled at 1, 5, 10 and 15 days after harvest. For each treatment and sampling date, the same section from eight different inflorescences were combined, frozen by liquid nitrogen, and kept at -20°C until used to determine total ethanol soluble sugar and sucrose.

Sugar extraction and determination from frozen tissues were as previously described (Section 2.3.4, Appendix B, C). For each section, the extractions were conducted repeatedly, three times from replicated tissues. Results were expressed as means from the three different extractions (n = 3). Sugar units were represented as glucose equivalent unit (for total ethanol soluble sugar) and sucrose equivalent unit (for sucrose).

6.2.3. Enzyme activities

Samples were taken on 6 different dates assigned as Day 0 (before hot water treatment), and 1, 4, 7, 10, and 13 days after the hot water treatment. For each sampling date, tissues from 15 individual inflorescences were taken and combined for each treatment. Sugar and four different enzyme activities (SPS, SS, AI and NI) were determined in two different tissues: bracts and stem sections. Bracts from the top portion of the inflorescence were selected to represent the bract sample, because senescence symptoms always developed in these tissues. The middle 15 cm of the stem was selected as representative of the whole stem. For each sample, tissues were separated for sugar extraction and dry weight, whereas the rest of the tissues was frozen in liquid nitrogen for enzyme extraction. Dry weight of the tissue was determined by drying tissue sample (2 to 3 g) in the oven (50-60°C) for at least 14 days. Sugar extraction was carried out on fresh tissues as previously described (Section 2.3.4). Total ethanol soluble sugar and sucrose were determined using phenol-sulfuric acid method (Dubois *et al.*, 1956), and reducing sugar by the 2-cyanoacetamide solution in the presence of 1.0 M borate buffer, pH 9.0 (Honda *et al.*, 1982). Detailed procedure for sugar extraction and determination are summarized in Appendix A and C.

Enzyme extraction: Enzymes were extracted and assayed for their activities with a modification of the method developed for rose (Khayat and Zieslin, 1987), citrus leaf (Schaffer, 1986), potato, bean and cassava (Sung *et al.*, 1989), and maize leaf (Huber and Huber, 1991).

Frozen tissues were ground to a powder in liquid nitrogen and homogenized (3 g) in 12 ml extraction buffer containing 50 mM Hepes-NaOH buffer (pH 7.5), 5 mM $MgCl_2$, 2 mM Na_2 EDTA, 2.5 mM dithiothreitol (DTT), 0.1% Triton X-100, 1% bovine serum albumin (BSA) and 2% polyvinylpolypyrolidone (PVPP). The extract was filtered through 4 layers of cheesecloth and centrifuged at 10,000 rpm (SA-600 rotor, Du Pont Instruments, Newtown C. T.) at 4°C for 20 min. The supernatant was desalted for 5 h at 4°C using Ultrafiltration membrane cones (137 x 32 mm for L x diameter, Amicon Inc., Beverly, MA) and assays for enzyme activities were conducted within 24 h.

Sucrose phosphate synthase (SPS) and sucrose synthase (SS) assay: SPS and SS were assayed as described by Khayat and Zieslin (1987). Aliquots of 70 µl of tissue extract were incubated at 37°C for 1 h with an equal volume of reaction mixture containing (70 µl) 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 5 mM NaF, 5 mM $Na_2MoO_4 2H_2O$, 15 mM UDP-glucose, 15 mM fructose-6-phosphate, and 20 mM glucose-6-phosphate. For SS activity, the fructose-6-phosphate and glucose-6-phosphate were replaced with fructose and DI water, respectively.

After 1 h of incubation period, the reaction was terminated by the addition of 70 µl of 1 N NaOH and boiled in water bath for 10 min. After cooling in tap water, sucrose product was determined colorimetrically at time zero and after 1 h incubation, using anthrone solution (Huber and Huber, 1991; Yemm and Willis, 1957). The determination of sucrose, instead of sucrose phosphate as a product of SPS activity, based on the presumption that SPS and sucrose phosphatase normally form enzyme complex *in vivo*, and sucrose phosphate formed by SPS is rapidly cleaved by sucrose phosphatase (Kruger,

1990). The determination of sucrose phosphate is elaborate and time consuming (Avigad, 1982), and is not suitable for the crude extract obtained in this study. The absorbance of the reaction was read at A_{620} nm using spectrophotometer (UV 160U, Shimadzu Corporation, Kyoto, Japan). Control for each assay contained all components except the tissue extract was replaced with extraction buffer. Enzymatic activity was determined by reference to the control assay and zero time activity subtracted from the 1 h incubation. Sucrose product was quantified by a standard curve using know amount of sucrose. Enzyme activity was expressed as μmole of sucrose formed per gram fresh weight per hour ($\mu mole \cdot g^{-1} \cdot h^{-1}$).

Acid invertase (AI) and neutral invertase (NI) assay: Invertase activity was determined as described by Schaffer (1986) with some modification. Two different pHs were used to determine activity of the enzymes: pH 5.0 for AI, and pH 7.0 for NI (Sung *et al.*, 1989). Aliquots of 25 μl of tissue extract were incubated with equal volumes of 1.0 M sucrose and 75 μl of 100 mM phosphate citrate buffer (pH 5.0 for AI, pH 7.0 for NI) at 37°C for 1 h. After incubation period, reaction was terminated by adding equal volume of reagents used to determine reducing sugar: 125 μl of 1% 2-cyanoacetamide and 1 ml 100 mM borate buffer, pH 9.0. (Honda *et al.*, 1982). Reaction was terminated by placing tubes in a boiling water bath for 10 min. After cooling in tap water, absorbance of the reaction was read at A_{276} nm. Control for each assay contained all components except the tissue extract was replaced with the extraction buffer. Enzymatic activity was determined by reference to the control assay and zero time activity subtracted from the 1 h incubation. Reducing sugar product was quantified by a standard

curve using know amount of glucose. Enzyme activity was expressed as μmole of reducing sugar formed per gram fresh weight per hour ($\mu mole \cdot g^{-1} \cdot h^{-1}$). The protocol to extract and assay enzyme activities is summarized in Appendix D.

6.3. Results and discussion

6.3.1. Apoplastic and symplastic sugars

Apoplastic sugar was determined directly from sap centrifuged out of stem section, while the stem sections after centrifugation were frozen and symplastic sugar extracted. Total sugar was determined based on total volume of sap and tissue weight. The major source of sugar in red ginger stem was symplastic sugar, i.e. 98%, whereas sugar in the apoplast contributed only 2% to the total sugars found in whole section.

Total apoplastic sugar did not significantly change after harvest (Fig. 6.2A), whereas total symplastic sugar significantly decreased ($P = 0.0001$, Fig. 6.2B). Hot water treated inflorescences had significantly higher total sugar than did unheated inflorescences in the symplast ($P = 0.002$), but not in the apoplast. Sucrose content in the apoplast and symplast significantly decreased after harvest ($P = 0.0002$, Fig. 6.3A, and $P = 0.0001$, Fig. 6.3B). Hot water treated inflorescences had significantly higher sucrose content than did unheated inflorescences in the symplast ($P = 0.004$), but not in the apoplast. These data suggested that the hot water treatment significantly affected symplastic sugars but not apoplastic sugars.

Based on unheated inflorescences, apoplastic sucrose one day after harvest (0.31%) was almost at the same level as the total sugar (0.38%) found in the same

sample, suggesting that major sugar found in apoplast was sucrose. Sucrose is the major sugar form transported in many plant tissues (Kruger, 1990).

6.3.2. Sugars in different parts of inflorescence

Total sugar content in 8 different plant sections determined one day after harvest (Day1) varied significantly with locations ($P = 0.0001$, Fig. 6.4). Total sugar in both the top and the bottom bracts was significantly lower than that found in other parts (Fig. 6.4A). The bottom part of the rachis had the highest total sugar content (131 $mg \cdot g^{-1}$ DW). However, total sugar in different stem sections was not significantly different from each other. Total sugar found in the bracts did not significantly change within 15 days after harvest, while sugar in rachis and stem sections significantly decreased ($P = 0.0002$, Fig. 6.4B).

Sugar content decline in the stems rather than bracts over 15 days after harvest (Fig. 6.4B) suggested that sugar was translocated from stems (source) to the inflorescence bracts (sink). This translocation took place before senescence symptoms developed in the bracts. The significance of inflorescence stem as an energy reserve for the floral tissues after harvest may be an interpretation of increasing dry weight of freesia florets with longer stem lengths (van Meeteren *et al.*, 1995).

Total bract sugar on unheated control in this study declined slightly over 15 days after harvest (Fig. 6.4A; B), compared to the dramatically declined ca. 7 days after harvest in previous study (Fig. 5.4A). This difference may be a seasonal effect, as the inflorescences in previous study were harvested in warm season (August 1997) and

developed senescence symptoms earlier (within 10 to 14 days after harvest) than the inflorescences harvested in cool period (this study, January 1999), which did not develop senescence symptoms until 15 to 18 days after harvest. The seasonal effects on inflorescence vase life have already been reported for red ginger (Hara *et al.*, 1997; Hara *et al.*, 1996).

It was unclear why inflorescences harvested during the cool period, when production was low (Criley, 1996), lasted longer than inflorescences harvested during the warm season. Low production and slow-growth in cool period may associate with high rate of loading-sugar and energy reserves per inflorescence in the same plant. The level of energy reserves of red ginger varied with harvest season, and consequently affected inflorescence vase life. Total sugar content for bracts and stems determined one day after harvest in cool period (Fig. 6.4A) were 1.1 and 1.7 folds higher than those in summer (Fig. 5.4A), respectively. Other possibility for seasonal effect on flower vase life may involve in high temperatures during growth and development are associated with faster utilization of carbohydrates accumulated in plant tissues (Nowak and Rudnicki, 1990). In Hawaii, average day temperature reach 29° to 30°C in the warm season, compared to 23° to 24°C in the cool period (NESDIS, 1997).

Total sugar in top and bottom bracts of untreated inflorescences was not significantly decrease with time after harvest (Fig. 6.5A), compared to a slight decrease in the hot water treated inflorescences (Fig. 6.5B, P = 0.002 and 0.015 for the top and bottom bracts, respectively). However, a decrease in sugar content over time was faster in bottom part of the rachis and in most stem sections of unheated inflorescences (P =

0.0001, Fig. 6.6A) than hot water treated inflorescences (P = 0.0001, Fig 6.6B). In this study, all unheated and hot water treated inflorescences did not show senescence symptoms when samples were taken 15 days after harvest (data not presented). This result suggested that hot water treatment had a greater effect on stem sugar than bract sugars, and the changes in stem sugar may induce inflorescence senescence, as its degradation took place prior to bract sugar degradation.

6.3.3. Enzyme activities

Sugar content: Total sugar in the inflorescence bracts remained relatively constant for 15 days after harvest, and there was no significant difference between unheated and hot water treated inflorescences (Fig. 6.7A). This was similar to previous experimental results (Fig. 6.5). Total stem sugars significantly decreased with time for both unheated and hot water treated inflorescences (P = 0.0001, Fig. 6.7B). At the end of experiment (13 days after harvest), total sugar of unheated stems was only 14% of the original level, compared to 44% of hot water treated stems.

Sucrose remained relatively constant in the bract, and there was no significant difference between unheated and the hot water treated bracts (Fig. 6.8A). Stem sucrose significantly decreased with time after harvest for both unheated and hot water treated stems (P = 0.0001, Fig. 6.8B). At the end of experiment (13 days after harvest), untreated stems maintained 34% of the original sucrose, compared to 68% in hot water treated stems. The average sucrose content of hot water treated stems was significantly higher than unheated stems (P = 0.0002).

Reducing sugar in the bracts did not change after harvest and there was no significant difference between unheated and hot water treated inflorescences (Fig. 6.9A). Stem reducing sugar significantly decreased with time after harvest in both unheated and hot water treated stems (P = 0.0001, Fig. 6.9B). At the end of experiment, unheated stems maintained 75% of original reducing sugar content, compared to 70% in hot water treated stems. The average content reducing sugar in hot water treated stems was significantly higher than untreated stems (P = 0.004).

Sugars are known to be a protective agent against heat injury (Levitt, 1980) and protein denaturation (Belikov *et al.*, 1963). Level of sucrose and monosacharides have been reported to increase when different leaves were exposed to a short period of heat stress (10 seconds at 50°C). However, with extended period of heat stress at moderate temperature (18 h at 37.5°C), the levels of most sugars in *Dactylis glomerata* declined (Zavadskaya, 1963). In muskmelon, heating fruit at 45°C for 3 h before storage at 4°C for 18 days inhibits the decline of sucrose content at the end of storage period (Lingle *et al.*, 1987). The increase in sugar content after the heat treatment may simply result from a protective mechanism of the plant adaptation to the heat stress. Results confirmed that hot water treated stems had levels of all sugars higher than the unheated stems, whereas levels of sugars in the bracts were not significantly different. These results suggested that some process associated with sucrose metabolism may be heat sensitive.

Senescence symptoms developed at almost the same time for both unheated and hot water treat inflorescences (ca. 7 days after harvest), but the development progressed faster in unheated inflorescences than in heated inflorescences (Fig. 6.10A). Percentage

of stem discoloration (turned from pink to white or pale color), after 7 days of harvest, was very high in unheated, compared to no discoloration in hot water treated stems at the end of experiment (Fig. 6.10B). This result suggested that hot water helped to maintain high sugar content in stem sections, hence maintaining their good appearance longer than unheated stems. Higher sugar content in stem sections associated with longer inflorescence vase life.

Energy reserves were not the only cause for red ginger senescence, as there are three major symptoms developed during red ginger senescence classified as inflorescence drooping, center bract browning, and bract darkening (Chapter 3). However, most inflorescences used in these studies showed center bract browning rather than other senescence symptoms. It was conclude that the most likely cause for center bract browning in red ginger was the depletion of energy reserves starting from the stems. Energy reserves in the cut stem have been related to vase life for Freesia (Sytsema-Kalkman *et al.*, 1995; van Meeteren *et al.*, 1995), rose (Marissen and Brijn, 1995), and *Sandersonia aurantiaca* (Eason *et al.*, 1997).

SPS and SS activity: The activity of SPS in the bracts did not change after harvest (Fig. 6.11A), whereas that in the stems decreased with time (P = 0.002, Fig. 6.11B). Hot water treated bracts had significantly higher overall SPS activity than unheated bracts (P = 0.0003), but there were no significant differences in the stem SPS activities. The activity of SS was significantly decreased with time after harvest in both bracts (P = 0.0001, Fig. 6.12A), and stems (P = 0.001, Fig. 6.12B). Hot water treated

bracts had higher SS activity than unheated bracts ($P = 0.001$), but the SS activity in the stems was not significantly different.

The increase in SPS and SS enzyme activities after hot water treatment may have resulted from the plant tissues stressed by the heat treatment (Feldman *et al.*, 1963; Levitt, 1980). Hence, heat stress tissues maintain their enzymatic reaction longer than non-heated tissues. The activities of SS and neutral invertase are also higher in heated muskmelon (45°C for 3 h) stored at 4°C for 18 days (Lingle *et al.*, 1987). However, Lingle et al. (1987) did not find any relationship between enzyme activities and sugar contents in the mesocarp tissues.

In red ginger, the relationship between sugar content and enzyme activity in different plant parts was not conclusive. In flower bracts, overall activities of SPS (Fig. 6.11A) and SS (Fig. 6.12A) were higher in hot water treated bracts than in the unheated control, whereas average sugar contents in the bracts (Fig. 6.7A; 6.8A; 6.9A) did not significantly differ. Hot water treated stems had total sugars (Fig. 6.7B) and sucrose (Fig. 6.8B) significantly higher than unheated stems, but both SPS (Fig. 6.11B) and SS (Fig. 6.12B) activities were not significantly different.

The lack of relationship between sugar contents and enzyme activities in this study could be due to differences between *in vitro* and *in vivo* activity due to the compartmentation and other metabolite levels (Lingle *et al.*, 1987). Alternatively, the colorimetric method used to determine sugar contents may not be suitable to distinguish the different types of sugar presented in the plant tissues. Finally, other factors, such as respiration rate and metabolic activity rate, rather than enzyme activity *per se*, may affect

sugar contents in the plant tissues. It is known that sugar is one of respiratory substrates for tissue respiration (Coorts, 1973), and after plant tissues are exposed to heat treatment, the respiration rate can drop to a lower level than in unheated tissues (Lurie and Klein, 1990). Nevertheless, during senescence, both anabolic and catabolic reactions take place (Brady, 1988). Therefore, sugar may also be withdrawn from internal sources for anabolic and catabolic activities.

Invertase activity: Invertase activity was not successfully determined possibly due to an inhibitor as reported for carnation petals (Halaba and Rudnicki, 1988). Further experiments are needed to develop a suitable protocol to extract and assay invertase activity in red ginger tissues.

6.4. Summary

The concentration of different sugars in different parts of the inflorescence was determined. Most stem sugar (98%) was located in the plant tissues (symplast), whereas the rest was attributed to apoplastic sugar. The sugar content was highest in rachis, and lowest in the inflorescence bracts. Significant changes in sugar content occurred in the stems after harvest. Hot water treated inflorescence maintained a higher level of sugar in the stems compared to unheated inflorescence ($P = 0.0001$), this may have delayed subsequent senescence development. There was no relationship between SPS and SS activities and the content of any sugar found in this study, whereas invertase activity was not detected. This finding suggested that factors other than sugar metabolic enzyme activity *per se*, may be involved in the postharvest sugar changes associated with the hot

water treatment. Other possible mechanisms could include the control of respiration rate either by metabolic enzymes or physical change of stomatal movement.

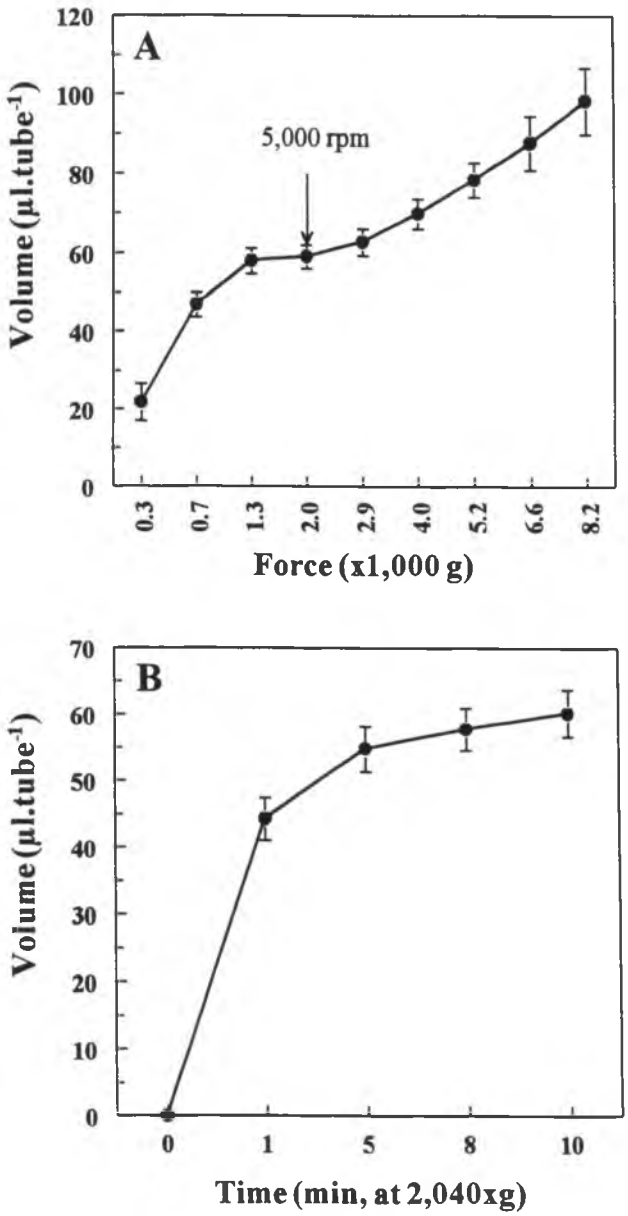


Figure 6.1. Sap accumulated from 1.5 cm stem sections after 15 min centrifugation at different centrifugal forces (A), and at 2,040 x g for different times (B). Each data point represents the mean from 12 sections.

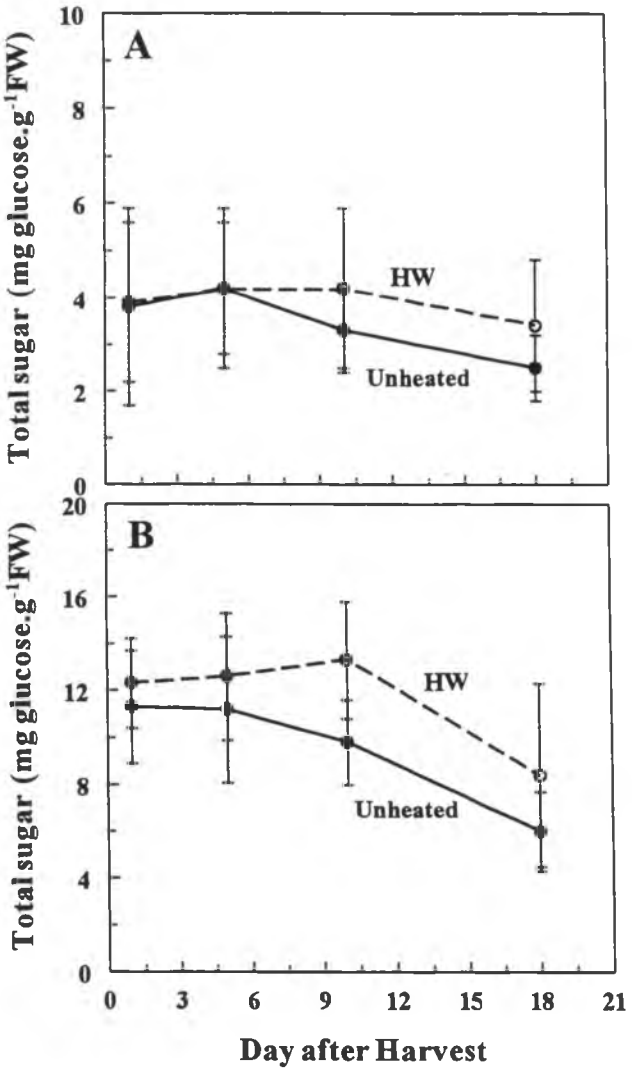


Figure 6.2. Changes in apoplastic (A) and symplastic (B) total ethanol soluble sugar in unheated (•) and hot water treated (o) stem sections. Samples were taken at 1, 5, 10 and 18 days after harvest. Each data point represents the mean from 12 sections. Unit was expressed as milligram glucose per gram fresh weight of the sample ($mg \cdot g^{-1}FW$).

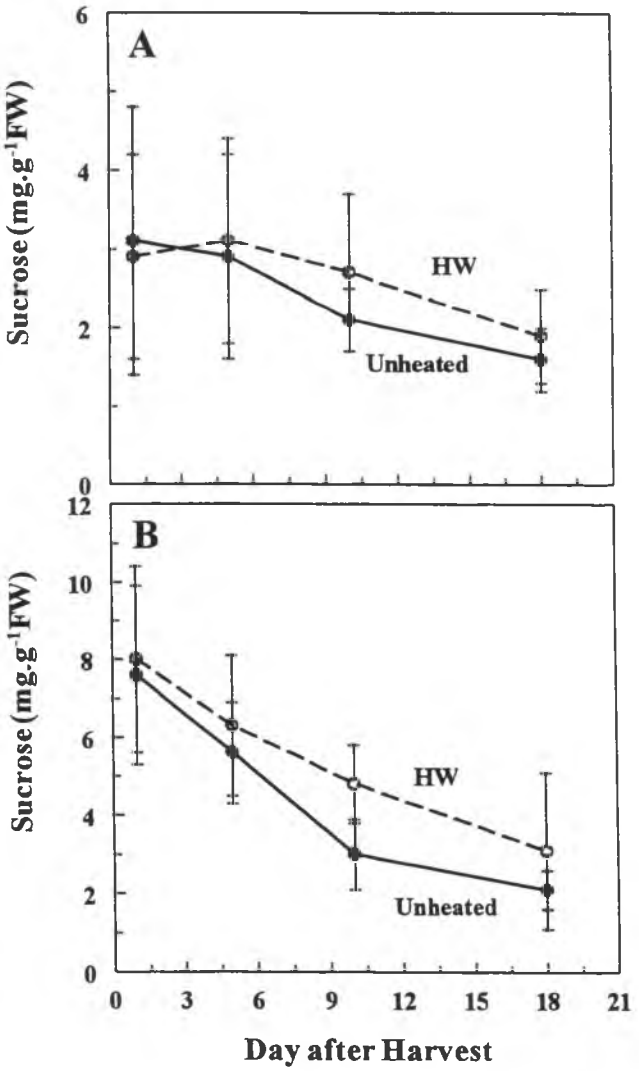


Figure 6.3. Changes in apoplastic (A) and symplastic (B) sucrose in unheated (•) and hot water treated (o) stem sections. Samples were taken at 1, 5, 10 and 18 days after harvest. Each data point represents mean the from 12 sections. Unit was expressed as milligram sucrose per gram fresh weight of the sample (mg sucrose·g^{-1}FW).

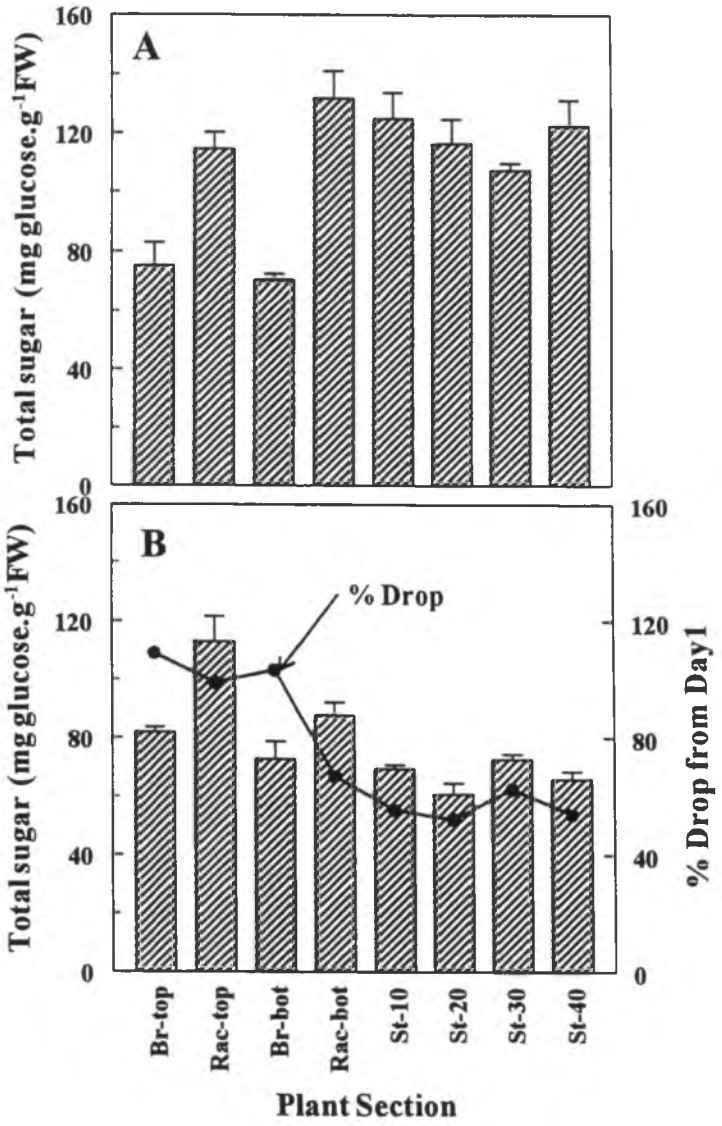


Figure 6.4. Total ethanol soluble sugar in different inflorescence parts 1 day (A), and 15 days (B) after harvest. For each part, sample was combined from 8 inflorescences and sugar extractions were performed separately 3 times. In (B), the percentage change from Day 1 to Day 15 is also plotted (•).

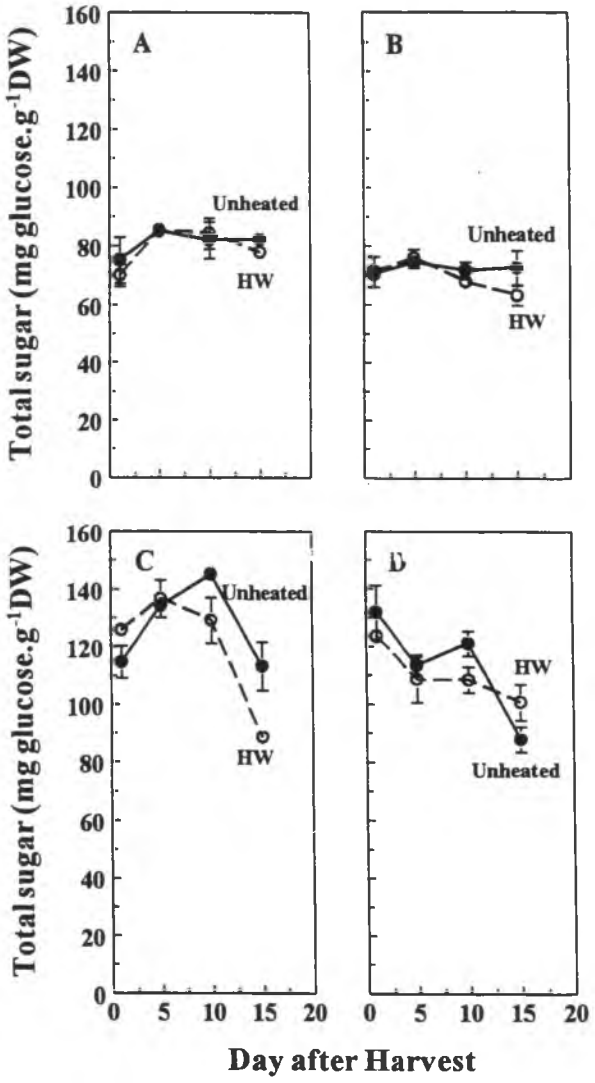


Figure 6.5. Total sugar in top bract (A), bottom bract (B), top rachis (C), and bottom rachis (D) of unheated (•) and hot water treated (o) inflorescences. Samples were taken at 1, 5, 10 and 15 days after harvest. For each sampling date, samples were combined from 8 inflorescences, and sugar extractions were performed separately 3 times. Unheated and hot water treated inflorescences were harvested one week apart from each other due to inflorescence availability.

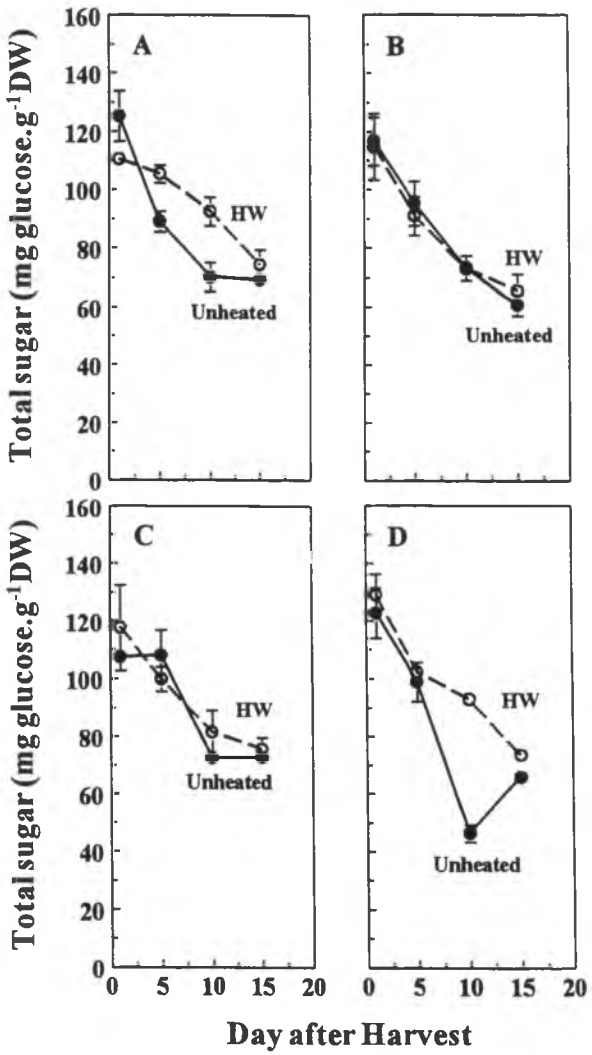


Figure 6.6. Total sugar in stem sections cut at 10 cm (A), 20 cm (B), 30 cm (C), and 40 cm (D) of unheated (•) and hot water treated (o) inflorescences. Samples were taken at 1, 5, 10 and 15 days after harvest. For each sampling date, samples were combined from 8 inflorescences, and sugar extractions were performed separately 3 times. Unheated and hot water treated inflorescences were harvested one week apart from each other due to inflorescence availability.

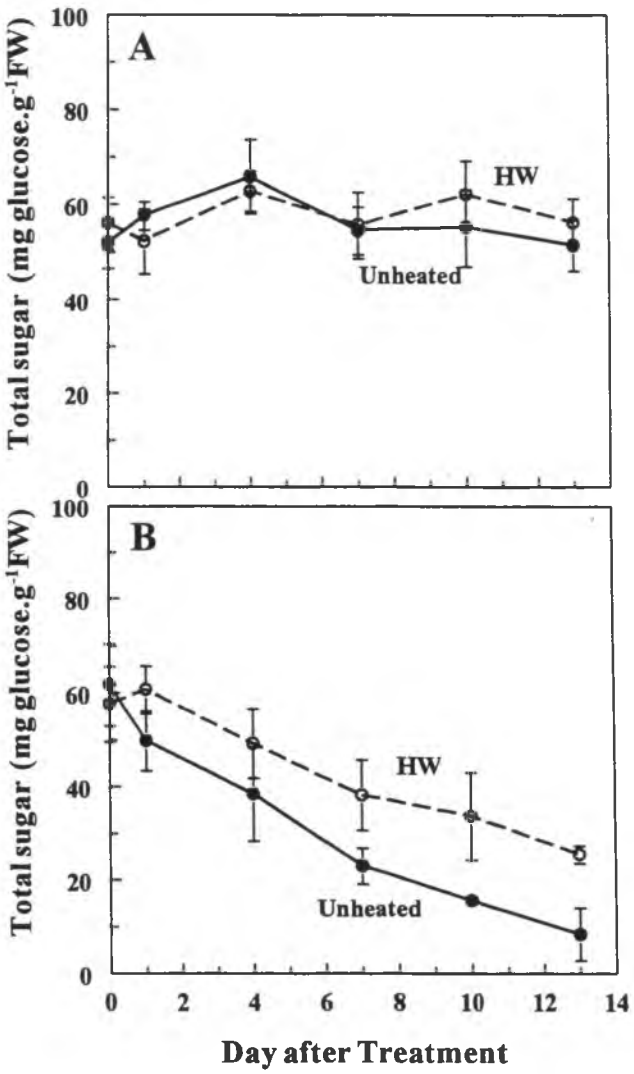


Figure 6.7. Changes in bract (A) and stem (B) total sugar in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10 and 13 days after treatment. For each sampling date, samples were combined from 15 inflorescences, and sugar extractions were performed separately 3 times.

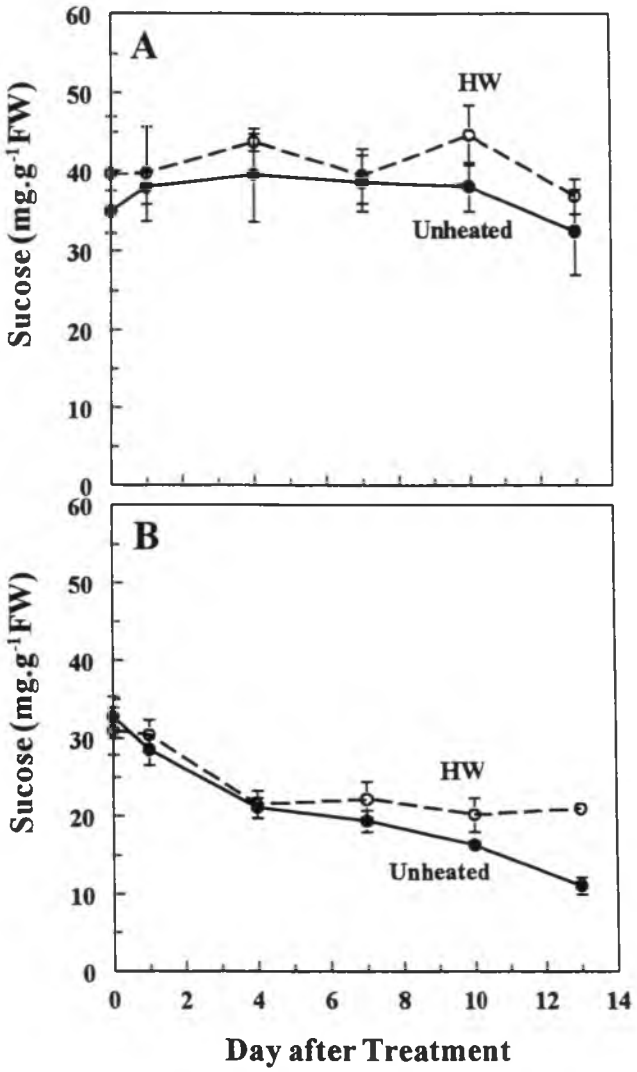


Figure 6.8. Changes in bract (A) and stem (B) sucrose in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10 and 13 days after treatment. For each sampling date, samples were combined from 15 inflorescences, and sugar extractions were performed separately 3 times.

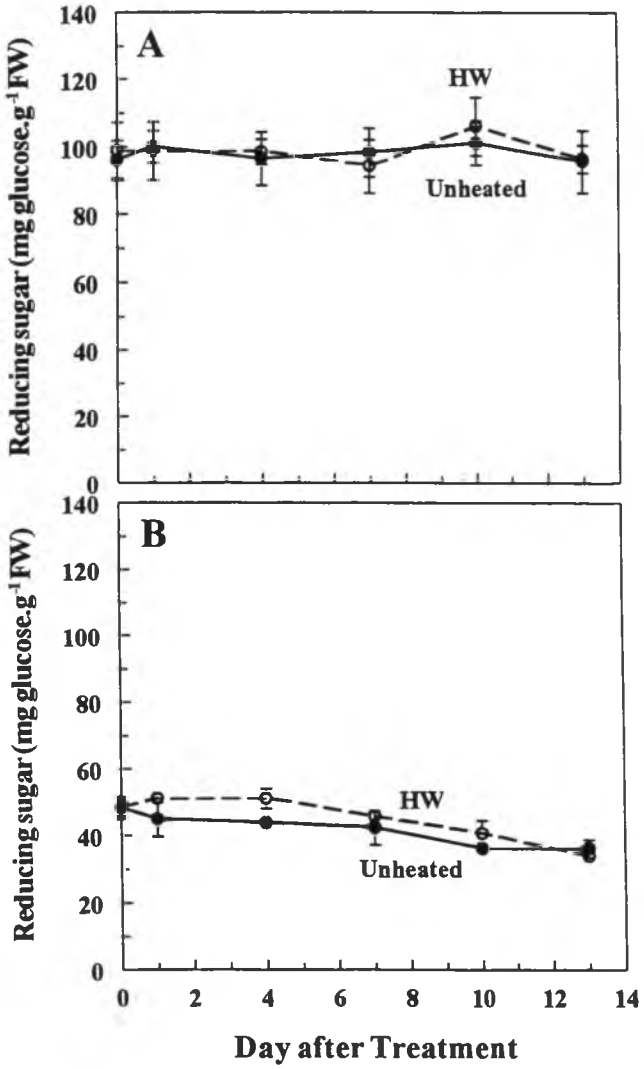


Figure 6.9. Changes in bract (A) and stem (B) reducing sugar in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10 and 13 days after treatment. For each sampling date, samples were combined from 15 inflorescences, and sugar extractions were performed separately 3 times.

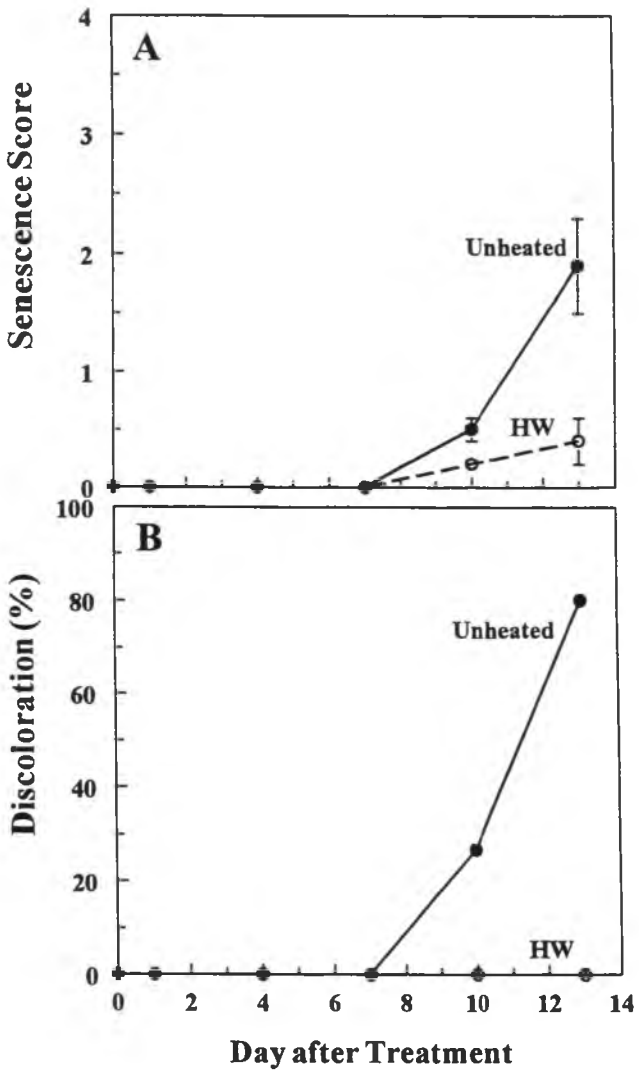


Figure 6.10. Bract senescence (A) stem discoloration (B) in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10, and 13 days after treatment. Each data point represents the mean from 15 inflorescences.

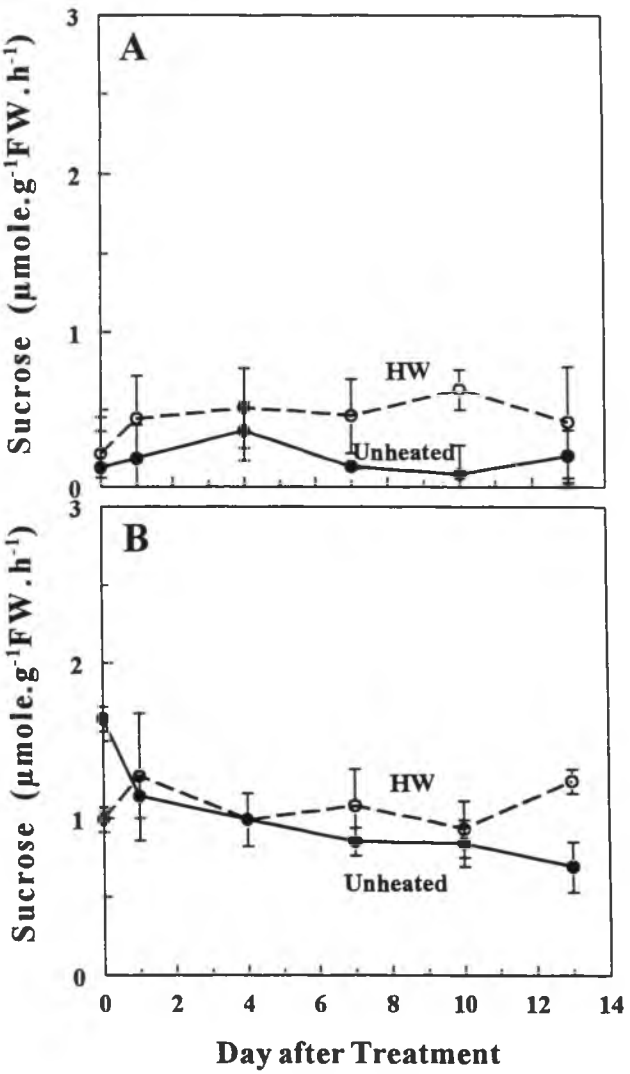


Figure 6.11. Bract (A) and stem (B) sucrose phosphate synthase (SPS) activity in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10 and 13 days after treatment. For each sampling date, samples were combined from 15 inflorescences, and enzyme extractions were performed separately 3 times. Enzyme activity was expressed as µmole of sucrose synthesis per gram fresh weight per hour (µmole sucrose·g^{-1}FW·h^{-1}).

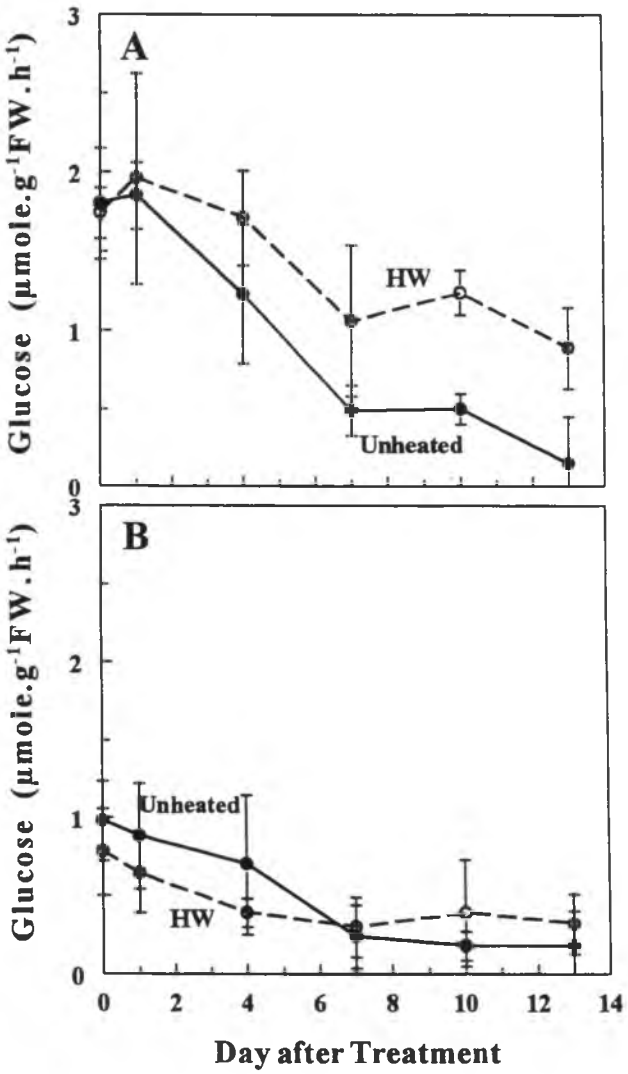


Figure 6.12. Bract (A) and stem (B) sucrose synthase (SS) activity in unheated (•) and hot water treated (o) inflorescences. Data were taken at 0, 1, 3, 7, 10 and 13 days after treatment. For each sampling date, samples were combined from 15 inflorescences, and enzyme extractions were performed separately 3 times. Enzyme activity was expressed as µmole of glucose synthesis per gram fresh weight per hour (µmole glucose·g^{-1}FW·h^{-1}).

CHAPTER 7

CONCLUSION

Postharvest physiology of many commercial tropical cut flowers, including red ginger, have not been studied in detail, despite being well known as exotic flowers, used throughout the world. Understanding of inflorescence characteristics and postharvest handling system could help to maintain postharvest quality and extend inflorescence vase life. None of inflorescence characteristic (maturity, stem diameter, stem length. and attached leaf to the stem) significant affects vase life, but application of benzyladenine (BA, 200 ppm) significant extend vase life. Irrespective of whether packed wet or dry, a plastic liner inside the cardboard box was crucial to maintain vase life.

Two major postharvest factors appeared to be associated with red ginger senescence: the water balance of the cut stem and the level of stored energy reserves. This conclusion was based on the major symptoms developed during red ginger senescence: inflorescence wilting, and center bract browning. These two senescent symptoms represented more than 90% of the symptoms developed in red ginger during senescence. While the relationship between water balance of the cut stem and vase life of the red ginger was not been established in this study, a positive relationship did exist between sugar content of the cut stem and the flower vase life. Neither ethylene nor the total number of stem microorganisms had an effect on triggering senescence in red ginger. These findings suggested that further improvement of the postharvest handling system for red ginger should concentrate on factors affecting water balance and energy

level of the cut stem, rather than those that alter ethylene production or tissue sensitivity, and the number of total microorganisms.

A suitable hot water treatment that extending vase life was preconditioning at 40°C for 15 min and then treatment at 50°C for 12 min, with a 1 h intervening period (Chapter 4). However, application of this recommended hot water treatment may induce more damage to the inflorescence bracts in cool season than in summer due to low thermotolerance in cool season. The exposure time in cool season, therefore, should be reduced (i.e. 50°C for 10 min), to prevent heat damage. The response of red ginger inflorescence to hot water treatment was dependent on seasonal factors and cultivars. Therefore, the generalized hot water treatment (50°C for 12 min) developed for other commodities may not be directly transferable.

Hot water treatment could extend the vase life via two major postharvest factors: lowering respiration rate during aging, and maintaining high level of sugar content of the red ginger inflorescences. No relationship was found between the activities of sugar metabolic enzymes (SPS and SS) and content of any sugar after the hot water treatment. Invertase activity was not detected. It was believed that factors other than sugar metabolic enzyme activities *per se* affected the postharvest sugar content of the red ginger. Other factors could include the respiratory enzyme activities, and changes in stomatal movement.

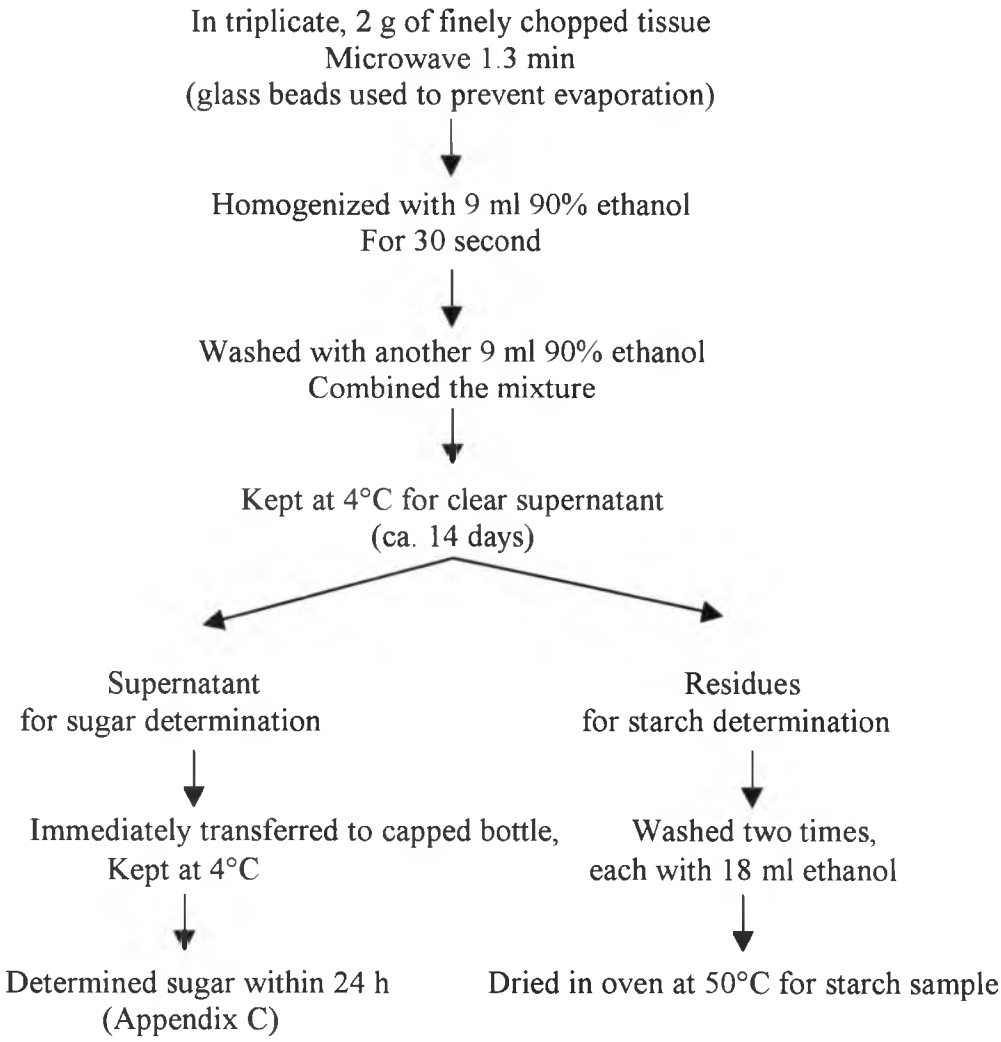
Besides the effects on postharvest physiological factors, hot water treatment had been proven to suppress negative geotropism developed in red ginger inflorescence. The delayed geotropic response can be maintained for up to 7 days.

Based on these studies, the following recommendation can be made for commercial practices:

1. Inflorescences should be harvest at mature stage (at least 2/3 open stage).
2. After harvest, inflorescences should be washed in tap water containing detergent to remove insect contamination and field heat.
3. Despite seasonal variation in the effecting of hot water treatment (preconditioning at 40°C for 15 min, and then hot water treatment at 50°C for 10 min (winter) to 12 min (summer)) to extend vase life. The treatment should be applied as it suppressed geotropic response during transportation.
4. Inflorescences should be sprayed with 200 ppm of BA before shipping.
5. Inflorescences can be packed wet (moistened newspaper) or dry (dry newspaper), but the plastic liner (20 μm thickness) in the cardboard box should not be omitted.
6. After packing, horizontally storage and shipping should not be any problem, for at least 7 days following heat treatment.
7. The application of chemicals to control ethylene synthesis and action was not effective.
8. Recommendations on the use of sucrose in the holding solution cannot be made as our results (2% w/v sucrose holding solution increased vase life) were on the stems without leaf sheaths.

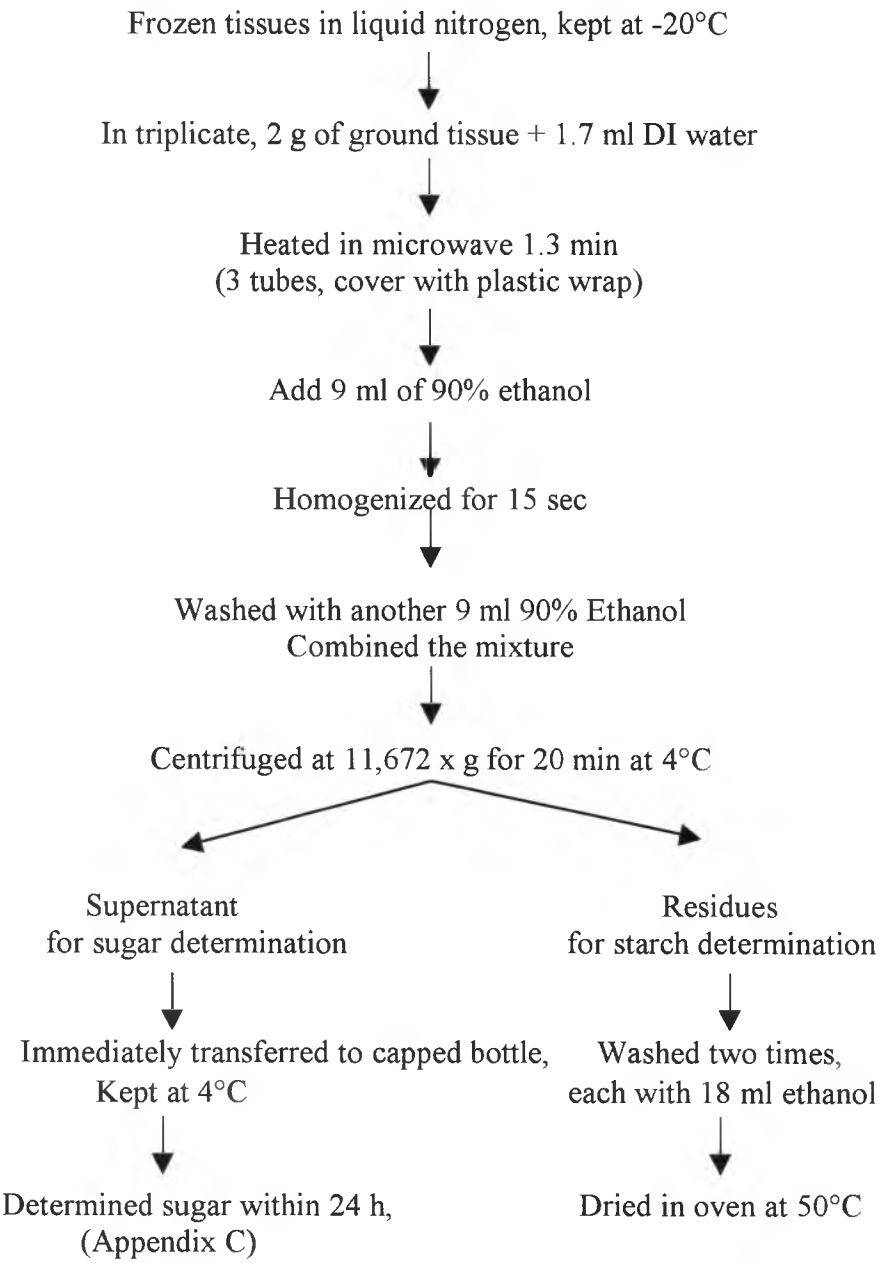
APPENDIX A

PROTOCOL USED TO EXTRACT SUGAR FROM FRESH TISSUES



APPENDIX B

PROTOCOL USED TO EXTRACT SUGAR FROM FROZEN TISSUES



APPENDIX C

DETERMINATION OF SUGAR BY COLORIMETRIC METHOD

Total sugar (Sample vol. = 100 µl)

25 µl tissue extract + 75 µl 90% ethanol, mix well

↓

Add 100 µl 5% Phenol, and 1 ml of H_2SO_4
Mix well by vortex

↓

Stand 20 min at room temperature, Read at A_{490} nm
(Standard curve using glucose, unit = mg glucose·gDW^{-1})

Sucrose (Sample vol. = 50 µl)

50 µl tissue extract + 50 µl 30% KOH, mix well

↓

Boil in water bath 10 min, cool down 10 min in tap water

↓

Add 100 µl 5% Phenol, and 1 ml of H_2SO_4
Mix well by vortex

↓

Stand 20 min at room temperature, Read at A_{490} nm
(Standard curve using sucrose, unit = mg sucrose·gDW^{-1})

Reducing Sugar (Sample vol. = 100 µl)

25 µl tissue extract + 75 µl 90% ethanol, mix well

↓

Add 100 µl 1% 2-cyanoacetamide

↓

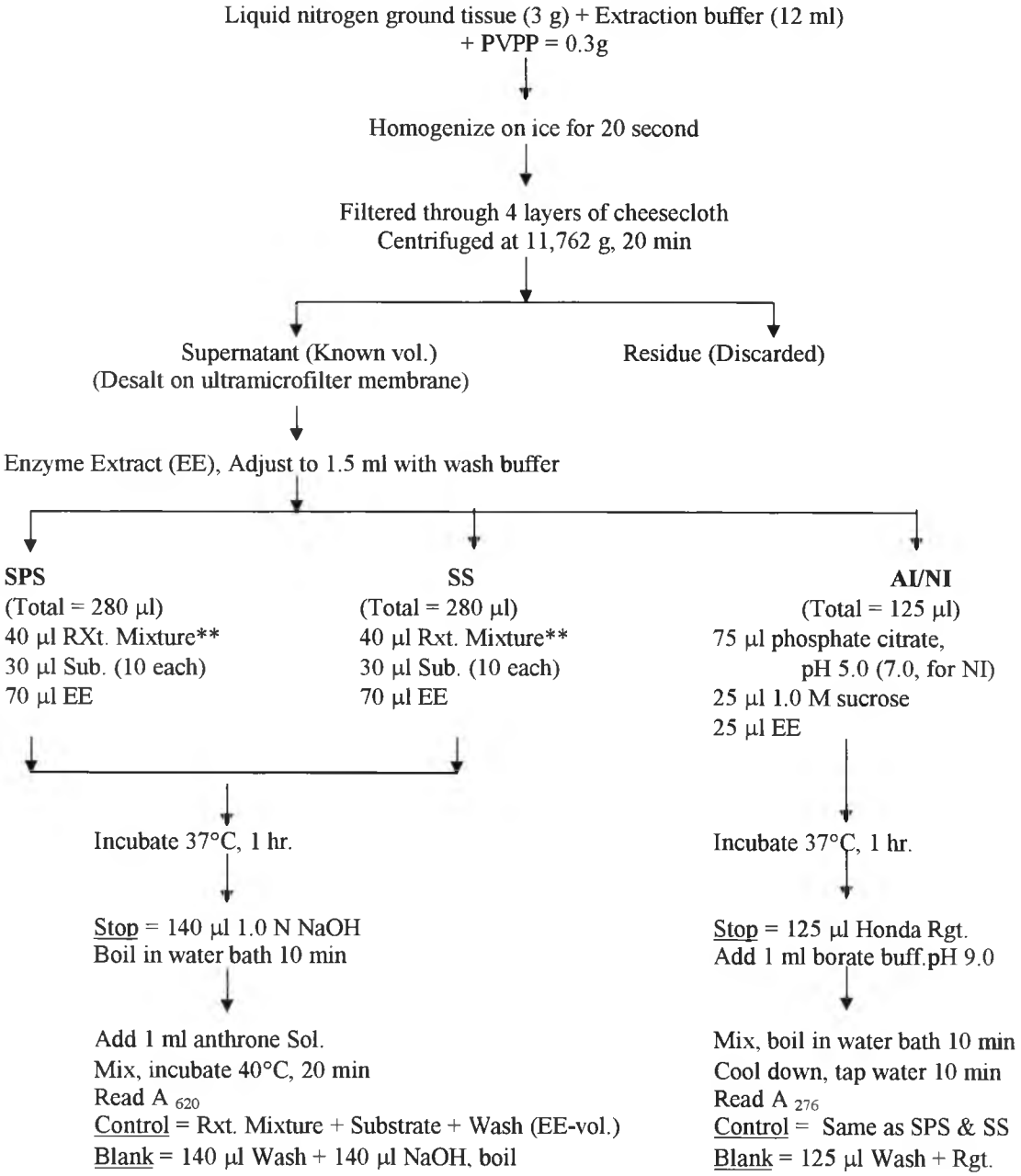
Add 1 ml of 0.1 M Borate buffer, pH 9.0

↓

Boil in water bath 10 min, cool down in tap water 10 min, Read at A_{276} nm
(Standard curve using glucose, unit = mg glucose·gDW^{-1})

APPENDIX D

EXTRACTION AND ASSAYS FOR SPS, SS, AI, AND NI



Note: 1. Read all sample against blank, including the control (2 reading/sample)
2 Subtract control from enzyme reading to get the activity at specific time
3. Subtract the activity at time zero from that of 1 hr incubation to get net activity
** Detail in text (Chapter 6).

APPENDIX E

WATER BALANCE COMPONENTS AND SENESCENCE SCORE

Infl. No.	day	loss	uptake	balance	score	Infl. No.	day	loss	uptake	balance	score
1	1	6.7	6.7	-0.1	0.0	6	1	5.0	4.8	-0.2	0.0
1	2	5.5	5.5	0.1	0.0	6	2	3.6	3.7	0.3	0.0
1	3	5.2	5.3	0.1	0.0	6	3	3.6	3.7	0.3	0.0
1	4	5.2	5.3	0.1	0.0	6	4	3.5	3.4	-0.2	0.0
1	5	5.2	5.3	0.2	0.0	6	5	3.3	3.4	0.2	0.0
1	6	5.2	4.8	-1.1	0.0	6	6	3.2	3.3	0.1	0.0
1	7	4.8	4.8	0.0	0.0	6	7	3.1	3.1	-0.1	0.0
1	8	4.8	4.7	-0.2	0.0	6	8	3.2	3.1	-0.3	0.0
1	9	4.5	4.4	-0.3	1.0	6	9	2.9	2.8	-0.2	0.0
1	10	4.8	4.5	-0.5	1.0	6	10	3.0	3.0	0.1	0.5
1	11	4.3	4.1	-0.5	2.0	6	11	2.9	2.7	-0.4	0.5
1	12	4.6	3.8	-2.1	3.0	6	12	2.9	2.4	-1.0	1.5
2	1	4.8	5.2	0.5	0.0	7	1	4.7	4.4	-0.4	0.0
2	2	3.7	4.2	0.9	0.0	7	2	3.3	3.5	0.4	0.0
2	3	3.8	4.1	0.6	0.0	7	3	3.4	3.5	0.2	0.0
2	4	3.8	3.8	0.1	0.0	7	4	3.4	3.3	-0.1	0.0
2	5	3.7	3.8	0.1	0.0	7	5	3.2	3.4	0.4	0.0
2	6	3.8	3.6	-0.4	0.0	7	6	3.1	3.1	0.2	0.0
2	7	3.4	3.3	-0.2	0.0	7	7	3.0	2.9	-0.2	0.0
2	8	3.4	3.4	0.0	0.5	7	8	3.1	2.9	-0.3	0.5
2	9	3.2	3.2	-0.1	1.0	7	9	2.9	2.8	-0.2	1.0
2	10	3.4	3.2	-0.5	1.0	7	10	3.0	2.7	-0.7	1.5
2	11	3.3	3.0	-0.6	1.0	7	11	3.2	2.5	-1.3	2.0
2	12	3.3	2.7	-1.4	2.5	7	12	3.3	2.2	-2.9	3.0
3	1	4.1	4.1	-0.1	0.0	8	1	5.3	5.3	0.0	0.0
3	2	3.1	3.3	0.4	0.0	8	2	4.1	4.2	0.2	0.0
3	3	3.1	3.4	0.5	0.0	8	3	4.0	4.2	0.4	0.0
3	4	3.1	3.2	0.3	0.0	8	4	4.0	4.1	0.2	0.0
3	5	3.0	3.2	0.3	0.0	8	5	4.0	4.0	0.1	0.0
3	6	2.9	3.0	0.4	0.0	8	6	3.8	3.8	0.2	0.0
3	7	2.8	2.7	-0.1	0.0	8	7	3.7	3.5	-0.4	0.0
3	8	2.9	2.9	0.1	0.0	8	8	3.8	3.7	-0.2	0.0
3	9	2.6	2.6	0.0	0.5	8	9	3.6	3.4	-0.4	1.0
3	10	2.8	2.8	-0.1	0.5	8	10	3.8	3.4	-1.0	2.0
3	11	2.8	2.7	-0.2	1.0	8	11	3.8	2.8	-2.3	2.5
3	12	2.8	2.5	-0.7	1.0	8	12	4.1	2.7	-3.7	4.0
4	1	4.1	4.0	-0.2	0.0	9	1	4.4	4.4	0.0	0.0
4	2	2.9	3.1	0.5	0.0	9	2	3.2	3.4	0.4	0.0
4	3	3.0	3.2	0.4	0.0	9	3	3.2	3.2	0.1	0.0
4	4	3.1	3.1	0.0	0.0	9	4	3.1	2.9	-0.4	0.0
4	5	3.0	3.1	0.2	0.0	9	5	3.0	2.9	-0.2	0.0
4	6	2.9	2.5	-0.9	0.0	9	6	2.8	2.7	-0.2	0.0
4	7	2.8	2.5	-0.4	0.0	9	7	2.7	2.5	-0.3	0.0
4	8	2.9	2.7	-0.4	0.5	9	8	2.8	2.5	-0.5	0.0
4	9	2.8	2.5	-0.5	1.5	9	9	2.6	2.3	-0.6	1.0
4	10	3.2	2.4	-1.4	2.5	9	10	2.8	2.1	-1.5	2.0
4	11	3.7	2.0	-3.3	3.0	9	11	3.2	1.8	-3.0	2.5
4	12	3.6	2.0	-3.8	4.0	9	12	3.5	1.6	-5.1	4.0

APPENDIX E

WATER BALANCE COMPONENTS AND SENESCENCE SCORE (Continue)

Infl. No.	day	loss	uptake	balance	score	Infl. No.	day	loss	uptake	balance	score
5	1	6.7	7.0	0.4	0.0	10	1	4.2	4.2	0.0	0.0
5	2	5.3	5.5	0.5	0.0	10	2	3.1	3.4	0.5	0.0
5	3	5.3	5.3	0.0	0.0	10	3	3.2	3.4	0.4	0.0
5	4	5.2	5.1	-0.2	0.0	10	4	3.1	3.2	0.2	0.0
5	5	5.1	4.8	-0.6	0.0	10	5	3.1	3.1	0.1	0.0
5	6	4.7	4.1	-1.5	0.0	10	6	2.9	3.0	0.2	0.0
5	7	4.6	4.3	-0.6	0.0	10	7	2.9	2.8	-0.2	0.0
5	8	4.5	4.3	-0.5	0.0	10	8	2.9	2.8	-0.2	0.5
5	9	4.1	3.7	-0.8	0.5	10	9	2.8	2.6	-0.3	1.0
5	10	4.0	3.4	-1.1	1.5	10	10	2.9	2.6	-0.6	1.5
5	11	4.0	2.8	-2.6	2.5	10	11	3.0	2.0	-2.1	2.5
5	12	4.2	2.6	-4.1	4.0	10	12	3.6	1.8	-4.6	4.0

APPENDIX F

PREHARVEST FACTORS, DAMAGE SCORE AND VASE LIFE

RAIN	7 DAY	14 DAY	21 DAY	30 DAY	VL-C	SCO-C	VL-HW	SCO-HW	DIF IN VL
10 Jan.	2.72	5.435	7.015	7.235	18.3	0	27	0.7	8.7
21 Feb.	0.125	1.385	2.31	5.68	20.5	0	17.4	0	-3.1
24 Apr.	0.205	0.395	0.6	0.94	15.3	0	31	0	15.7
1 May.	1.87	2.075	2.265	2.47	20.4	0	30.9	0.1	10.5
29 May.	0.12	0.155	0.56	2.925	19.5	0	34.3	0	14.8
3 July.	0.455	0.69	1.74	4.9	14.9	0	27.8	0	12.9
24 July.	0.49	0.61	0.97	1.585	18.8	0	19.7	0	0.9
13 Aug.	0.075	0.125	0.865	1.435	21.3	0	17.7	0	-3.6
17 Sept.	0.54	5.25	8.24	8.245	14.1	0	12.8	0	-1.3
15 Oct.	0.06	0.06	0.06	0.63	15.9	0	17.5	0	1.6
19 Nov.	18.9	26.22	26.385	27.69	12.9	0	18.6	0.8	5.7
3 Dec.	0.26	0.34	19.24	26.67	11.4	0	10.3	0.1	-1.1
MAX	1 DAY	3 DAY	5 DAY	7 DAY	14 DAY	21 DAY	30 DAY	VL	SCORE
10 Jan.	80.0	79.0	78.4	78.9	77.8	78.8	79.9	27	0.7
21 Feb.	70.0	69.0	68.4	68.0	71.6	73.1	74.6	17.4	0
24 Apr.	86.0	84.0	83.4	82.6	81.9	81.9	80.5	31	0
1 May.	68.0	78.0	80.0	81.6	82.1	81.8	81.9	30.9	0.1
29 May.	82.0	83.3	83.8	84.6	84.4	82.5	80.5	34.3	0
3 July.	88.0	86.7	84.8	84.4	83.8	83.6	83.6	27.8	0
24 July.	89.0	88.0	87.3	86.7	86.8	86.7	85.9	19.7	0
13 Aug.	87.0	87.3	87.4	87.1	86.9	87.4	87.1	17.7	0
17 Sept.	84.0	84.7	84.2	84.0	83.9	84.5	85.8	12.8	0
15 Oct.	87.0	87.3	87.8	88.3	86.8	86.5	86.3	17.5	0
19 Nov.	75.0	75.7	75.2	75.7	76.9	79.4	81.2	18.6	0.8
3 Dec.	81.0	79.7	80.8	80.6	80.6	79.0	79.2	10.3	0.1
MIN	1 DAY	3 DAY	5 DAY	7 DAY	14 DAY	21 DAY	30 DAY	VL	SCORE
10 Jan.	67.0	65.3	65.4	64.4	64.4	64.3	65.5	27	0.7
21 Feb.	60.0	57.0	57.8	58.1	60.3	61.3	62.6	17.4	0
24 Apr.	68.0	68.0	68.6	68.1	67.0	66.9	65.5	31	0
1 May.	62.0	65.7	66.8	67.3	67.7	67.1	67.0	30.9	0.1
29 May.	70.0	70.0	70.4	70.7	70.9	68.9	67.5	34.3	0
3 July.	70.0	70.7	70.6	70.6	70.4	70.5	70.5	27.8	0
24 July.	72.0	70.5	70.0	69.8	70.3	70.1	70.2	19.7	0
13 Aug.	74.0	73.7	72.0	71.7	71.6	71.5	71.0	17.7	0
17 Sept.	72.0	71.0	71.2	71.3	71.9	72.0	71.4	12.8	0
15 Oct.	71.0	72.7	72.0	72.0	70.6	70.2	70.7	17.5	0
19 Nov.	65.0	69.0	69.4	69.9	69.7	69.8	70.1	18.6	0.8
3 Dec.	69.0	69.0	68.4	68.7	68.2	68.8	69.1	10.3	0.1
MEAN	1 DAY	3 DAY	5 DAY	7 DAY	14 DAY	21 DAY	30 DAY	VL	SCORE
10 Jan.	73.5	72.2	71.9	72.8	71.8	72.0	73.0	27	0.7
21 Feb.	65.0	63.0	63.1	63.1	65.9	67.2	68.6	17.4	0
24 Apr.	77.0	76.0	76.0	75.4	74.4	74.4	73.0	31	0
1 May.	65.0	71.8	73.4	74.4	74.9	74.4	74.5	30.9	0.1
29 May.	76.0	76.7	77.1	77.6	77.6	75.7	74.0	34.3	0
3 July.	79.0	78.7	77.7	77.5	77.1	77.0	77.1	27.8	0
24 July.	80.5	79.3	78.6	78.3	78.6	78.4	78.1	19.7	0
13 Aug.	80.5	80.5	79.7	79.4	79.2	79.5	79.1	17.7	0
17 Sept.	78.0	77.8	77.7	77.6	77.9	78.3	78.6	12.8	0
15 Oct.	79.0	80.0	79.9	80.1	78.7	78.4	78.5	17.5	0
19 Nov.	70.0	72.3	72.3	72.8	73.3	74.6	75.6	18.6	0.8
3 Dec.	75.0	74.3	74.6	74.6	74.4	73.9	74.1	10.3	0.1

APPENDIX G

TAP WATER COMPOSITION

Location	Kaimuki Low Service	Beretania Low Service
Reginal head, feet	22.96	20.94
Specific conductance (micromhos at 25°C)	423	352
pH value	8.1	8.2
Turbidity	0	0
Color	0.3	0.3
In part per million (ppm)		
Dissolved oxygen	8.45	9.0
Free carbon dioxide	0	0
Silica	35	38
Calcium	8.8	10
Magnesium	11	11
Sodium	58	40
Potassium	2.6	3.8
Bicarbonate	77	79
Sulfate	15	9.3
Chloride	82	61
Fluoride	0.05	0.05
Nitrate	1.6	2.1
Phosphate	0.2	0.1
Iron	< 0.02	< 0.02
Manganese	< 0.02	< 0.02
Copper	< 0.02	< 0.02
Lead	< 0.02	< 0.02
Arsenic	< 0.01	< 0.01
Selenium	< 0.01	< 0.01
Chromium	< 0.01	< 0.01
Total dissolved solides	291	254
Alkalinity	63	65
Total hardness	67	70

Source: Board of Water Supply. City and County of Honolulu, Hawaii.

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